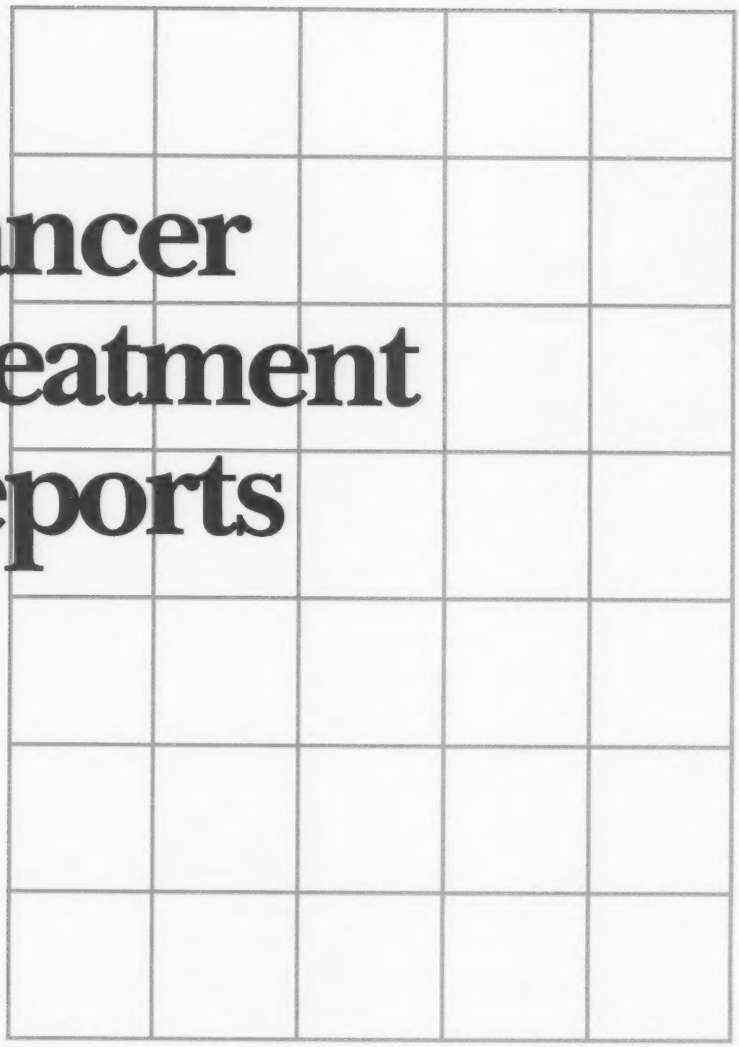

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Cancer Treatment Reports

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3. The study should not have an inevaluability rate for major endpoints of greater than 15%. Not more than 15% of eligible patients should be lost to follow-up or considered inevaluable for response due to early death, protocol violation, missing information, etc.

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9. The methods of statistical analysis should be described in detail sufficient that a knowledgeable reader could reproduce the analysis if the data were available.

**From* Methodologic Guidelines for Reports of Clinical Trials (Cancer Treat Rep 69:1-3, 1985).

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995 ANNOUNCEMENTS

Gallium Nitrate in Prostatic Cancer: Evaluation of Antitumor Activity and Effects on Bone Turnover[1,2]

Howard I. Scher,* Tracy Curley, Nancy Geller, David Dershaw, Eva Chan, Jerome Nisselbaum, Nancy Alcock, Phyllis Hollander, and Alan Yagoda[3]

Gallium nitrate, an agent known to inhibit bone resorption, was evaluated in patients with bidimensionally measurable hormone-refractory prostatic cancer. The starting dose was 200 mg/m^2 iv by continuous infusion over 7 days. Two patients (10%; 95% confidence limits, 0%–22%) achieved short partial remissions of 1 and 6+ months, while seven of 23 (30%; 95% confidence limits, 14%–52%) showed a diminution of bone pain. Serial indices of bone turnover including serum calcium, phosphorus, and urinary hydroxyproline excretion showed a significant decrease at the completion of the infusion which returned to baseline prior to the next cycle. The data suggest the effect on bone was too short to produce consistent improvement. Reasons for the dissociation of pain relief and antitumor activity are discussed. [Cancer Treat Rep 71:887–893, 1987]

Osseous metastases ultimately develop in > 90% of patients with metastatic prostatic cancer, and in 80% of the cases it is the sole site of metastatic spread (1,2). These can produce severe bone pain, pathologic fractures, and progress to epidural cord compression. In untreated cases, androgen ablation will produce clinical improvement and associated healing of bony disease in 60%–80% of patients (3). In hormone-refractory cases, objective healing of bone lesions is infrequent and the effects of systemic chemotherapy are often difficult to assess (4,5). In many cases, subjective improvement in bone pain is not accompanied by radiographic improvement of bone lesions, decreasing activity on radionuclide scan, or improvement of an abnormal biochemical parameter. These factors may in part explain the wide disparity in response rates reported for many chemotherapeutic agents in this disorder (3,6,7).

Increased bone turnover, as manifested by elevated urinary hydroxyproline excretion, has been reported in patients with prostatic cancer (8–11). Further, histomorphometric evaluation of bone metastases in patients with hormone-refractory disease has shown increased resorptive activity (12). Treatment with agents known to inhibit bone resorption, such as dichloromethylene diphosphonate, has been shown to produce improvement in bone pain in the absence of antitumor activity (13).

Gallium nitrate is an anhydrous salt of the naturally occurring group IIIa metal salt (14,15). Interest in the agent as a treatment for bone metastases stemmed from the observation that the compound can induce hypocalcemia when administered as a continuous infusion (16,17). Additional studies showed that gallium was incorporated into hydroxyapatite, making the crystals less soluble and less susceptible to dissolution (18). Inhibition of bone resorption occurs in the absence of toxic effects to bone cells (19).

An often insurmountable problem for evaluation of response to therapy of prostatic cancer is the paucity of bidimensionally measurable lesions that can be followed objectively. At Memorial Hospital, we have restricted trials to patients with bidimensionally measurable disease (5,20,21). This excludes many patients from entry into clinical trial. However, this is the only subgroup in whom meaningful response evaluations can be performed. To further evaluate the effect of gallium nitrate in prostatic cancer, a parallel investigation of the effects

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[3] Solid Tumor Service, Department of Medicine (*H. I. Scher, T. Curley, P. Hollander,* and *A. Yagoda*), Department of Biostatistics (*N. Geller* and *E. Chan*), Department of Medical Imaging (*D. Dershaw*), and Department of Clinical Chemistry (*J. Nisselbaum* and *N. Alcock*), Memorial Sloan-Kettering Cancer Center, New York, NY, and Department of Medicine (*H. I. Scher* and *A. Yagoda*), Cornell University Medical College, New York, NY.

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of gallium nitrate on bone turnover and a clinical trial in patients with bidimensionally measurable disease were undertaken at Memorial Hospital.

MATERIALS AND METHODS

Patients with hormone-resistant metastatic prostatic adenocarcinoma histologically confirmed by the Department of Pathology were considered. Entry requirements included a Karnofsky performance status (KPS) $\geq$ 50%, adequate hematologic status (wbc count $\geq$ 3000/mm^3, platelet count $\geq$ 200,000/mm^3), serum bilirubin $\leq$ 1.5 mg/dl, creatinine $\leq$ 2.0 mg/dl, BUN $\leq$ 30 mg/dl, clinically normal auditory function, creatinine clearance $>$ 50 ml/minute/1.73 m^2, and bidimensionally measurable disease. Measurable disease included lymph nodes palpable by physical examination, pulmonary nodules, retroperitoneal and pelvic masses on computerized tomographic (CT) scan, transabdominal ultrasound of the liver, or transrectal ultrasound of the prostate. Palpable tumor masses were measured in the largest perpendicular diameter by two independent observers. All roentgenographic material was evaluated independently (D. Dershaw). When final review did not confirm bidimensionally measurable disease, patients were reclassified as evaluable (eg, osseous metastases, unidimensional pleural based masses, prostatic enlargement, or elevated acid phosphatase) and considered separately.

Patients may have had prior chemotherapy with one single agent or combination, but may not have received chemotherapy or radiation therapy within 3 weeks of the start of the protocol. Prior to entry, all patients had a complete history and physical examination. Laboratory evaluation included an automated blood and platelet count, 12-channel screening profile, serum acid phosphatase, 5′-nucleotidase, C-terminal parathyroid hormone, 1,25-$(OH)_2$ vitamin D_3 and 25-OH vitamin D_3, osteocalcin, and serum creatinine. A 24-hour urine was evaluated for hydroxyproline, creatinine, calcium, and phosphorous. Hydroxyproline was assayed by a modification of the method of Goverde and Veenkamp (22). The resin was converted to the hydrogen form on the day of performance of the test and washed until the pH of the supernatant stabilized at approximately 3.3. To correct for differences in renal function, results were expressed as the ratio of hydroxyproline/creatinine (8,9). Osteocalcin was assayed by radioimmunoassay by the method of Lian et al (23) with a commercially available kit (Seragen, Boston, MA).

Radiographic examinations included a chest roentgenogram and (to evaluate for measurable disease) CT scan of the abdomen and pelvis or transrectal ultrasound. Roentgenograms and scans were repeated at 4–6-week intervals when used to evaluate measurable disease. Radionuclide bone scans with roentgenographic evaluation of abnormal areas were also obtained and repeated after 3-month intervals. Informed consent was obtained prior to initiation of therapy.

All patients were hospitalized during treatment. The starting dose of gallium nitrate was 200 mg/m^2 iv by continuous infusion over 24 hours for 7 days. Hydration was performed to maintain a urine output $>$ 2 L/day. Serum calcium and creatinine determinations were performed daily during treatment. Screening profiles, osteocalcin determinations, and hydroxyproline/creatinine ratios were repeated after the completion of the cycle (usually Day 8) and prior to the next cycle. Automated blood counts and platelet counts were obtained 10–14 days after treatment. Cycles were repeated every 28 days. Dose escalations were performed to 250 and 300 mg/m^2 iv over 24 hours for 7 days if only reversible grade $\leq$ 1 renal toxicity was observed. Therapy was discontinued if grade 3 (creatinine $>$ 5 $\times$ baseline) was observed. Other toxic effects were graded according to the World Health Organization (WHO) modified toxicity scale in use at Memorial Hospital. An adequate trial was defined as one cycle (7 days) of therapy for evaluation of toxicity or one cycle and 3 weeks of observation for response.

Response was assessed as previously described (5, 20,21). Patients who required radiation therapy for symptomatic bony metastases were considered to have progressed at the start of radiation therapy. Duration of response was measured from initiation of therapy until documentation of progression at any site.

STATISTICAL METHODS

Several biochemical parameters (calcium, phosphorus, albumin, acid and alkaline phosphatase, lactate dehydrogenase, C-terminal parathyroid hormone, 25-OH and 1,25-$(OH)_2$ vitamin D_3, osteocalcin, urinary calcium, and urinary hydroxyproline/creatinine ratios) were compared at three time points: prior to the first cycle of treatment, on Day 8 following treatment, and prior to the second cycle of treatment, using the Friedman test (24). When differences were found to be significant at the 0.05 level, pairwise comparisons were made, again using the Friedman test, this time at the 0.0167 level, because of multiple hypothesis tests.

RESULTS

A total of 23 patients were entered. After review of all available data, three (13%) were reclassified as evaluable. The clinical characteristics of patients with bidimensionally measurable and evaluable disease are listed in table 1. For the measurable disease group, the median age was 65 years and the median KPS was 70%. Three patients had received prior chemotherapy (one, methotrexate; one, doxorubicin; and one, cisplatin and etoposide). All had progressed on prior hormonal therapy, including orchiectomy in seven, diethylstilbesterol in six, or orchiectomy plus other hormones in seven. Fourteen patients had received prior radiation therapy to the following sites: prostate alone in two, prostate and other site in four, spine in six, and appendicular sites in two. The primary measurable indicator lesion included lymph nodes in 12 (three by physical examination and nine by CT scan); pulmonary lesion in two; hepatomeg-

TABLE 1.—Clinical characteristics*

Characteristic	Measurable	Evaluable
Median age in yrs (range)	65 (49–72)	66 (65–68)
Median Karnofsky performance status in % (range)	70 (50–90)	80 (60–90)
Prior chemotherapy	3 (15)	1 (33)
Prior radiation therapy		
None	6 (30)	2
Prostate	2 (10)	0
Prostate + other	4 (20)	1
Spine	6 (30)	1
Appendicular	2	0
Prior hormones		
Orchiectomy alone	7	1
Orchiectomy + diethylstilbestrol	4	2
Orchiectomy + other	3	0
Diethylstilbestrol alone	6	0
Primary indicator lesions		
Nodes		
Physical examination	3	
CT	9	
Pulmonary	2	
Sacral mass	1	
Prostate		
CT scan	3	
Sonogram	1	
Hepatic	1	

*Unless otherwise indicated, values = No. of patients (%).

aly by sonography in one; prostatic masses by CT scan in three and ultrasound in one; and a sacral mass by CT in one. In three patients with evaluable disease, two had had prior chemotherapy, three had an elevated alkaline phosphatase, two had an increased hydroxyproline/creatinine ratio, one had an elevated osteocalcin, and two had an elevated acid phosphatase. Baseline biochemical parameters included an elevated acid phosphatase in 86% (18 of 21 cases), increased alkaline phosphatase in 91% (21 of 23), increased hydroxyproline/creatinine ratio (> 5.0) in 77% (17 of 22), and elevated osteocalcin in 47% (eight of 17).

Of 20 patients with bidimensionally measurable disease, two patients (10%; 95% confidence limits, 0%–22%) achieved a partial response. One had regression of biopsy-proven cervical lymphadenopathy for 5+ months, but progressed with pulmonary nodules after 1 month. A second had healing of a presacral mass with associated osteolytic disease for 6+ months but progressed with epidural bone disease at a distant site (fig 1). A third achieved a minor response in hepatic disease on physical examination and hepatic ultrasound with normalization in the acid phosphatase from a baseline level of 3.0 to 0.5 U/L (normal, 0–0.6 U/L) for 3 months. Six patients (30%), including one with evaluable disease only, showed a > 50% decrease in acid phosphatase following treatment. Three additional patients had relief of bone pain and decrease in analgesic requirements with evidence of progression of soft tissue disease.

Of the evaluable patients, one developed symptomatic hypocalcemia requiring discontinuation of therapy, another had a decrease in acid phosphatase from 39 to 4.2 U/L associated with a decrease in bone pain, and the third patient experienced a decrease in bone pain without change in biochemical parameters. Overall, seven (30%) patients noted an improvement in bone pain.

Fifty-five cycles were administered to the 20 evaluable patients with measurable disease and 5.5 cycles to the three patients with evaluable disease. The median number of cycles per patient was three (range, one to five). Seventeen patients had the daily dose escalated to 250 mg/m^2 and five to 300 mg/m^2. One patient had a dose de-escalation from 250 mg/m^2 to 200 mg/m^2 because of transient pulmonary infiltrates which did not recur. Adverse reactions (table 2) included hypocalcemia (< 8.0 mg/dl) in 53% (12 of 23) of the cases, and hypophosphatemia (< 2.0 mg/dl) in 48% (11 of 23). Five

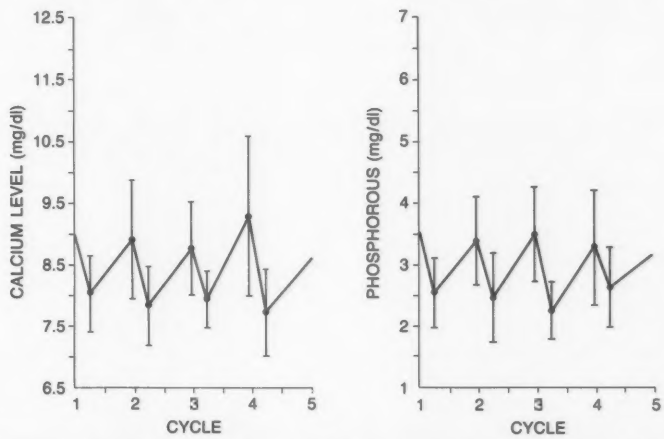

FIGURE 1.—Plot of sequential changes in calcium and phosphorus. Note decrease in levels which return to baseline following sequential cycles.

TABLE 2.—Toxic effects of gallium nitrate infusions

Toxic effects	No. of patients (%)
Median hemoglobin decrease in g/dl (range): −3.9 (−1.9–4.6)	
Increase in serum [creatinine] (mg/dl)	
< 0.4	18 (74)
0.5–1.0	2 (9)
≥ 1.1	3 (13)
Decrease in total serum [calcium] (mg/dl)	
< 1.0	4 (18)
1.1–2.4	12 (52)
≥ 2.5	7 (30)
Decrease in serum inorganic [phosphorous] (mg/dl)	
< 0.4	0 (0)
0.5–1.5	8 (34)
≥ 1.6	15 (66)

(22%) patients, two of whom were symptomatic, received calcium supplementation (which was administered when the serum calcium fell to < 7.5 mg/dl: one required discontinuation of the infusion on Day 5 and was not retreated. Renal toxicity occurred in three patients. One patient developed a transient reversible increase in the serum creatinine from 0.9 to 1.7 mg/dl. A second patient who developed fever on Day 6 of the fourth cycle of therapy was given gentamicin. The serum creatinine increased to 9.7 mg/dl and dialysis was required for 1 month. The creatinine subsequently returned to the baseline level and dialysis was discontinued. A third patient developed an increase in serum creatinine concentration to 2.5 mg/dl. This patient had developed clinical signs of sepsis and died. The death was not felt to be drug related.

Myelosuppression was not observed. Only one (4%) patient developed a transient decrease in platelet count to < 125,000/mm^3. A decrease in hemoglobin was observed. The mean decrease was 3.9 g/dl (range, 1.9–4.6 g/dl) and 11 patients required at least two units of blood during therapy. Gastrointestinal toxicity included transient nausea in three (13%) of the patients and diarrhea, which required symptomatic therapy, in three (13%). Evaluation in two cases showed evidence of both fat (abnormal ^{14}C breath test) and sugar (abnormal D-xylose) absorption. Vitamin D levels were also decreased. An intestinal biopsy revealed atrophic villi with a decrease in crypt height. These symptoms improved minimally with a lactose-free diet. The patient also developed dry scaling skin, felt to be treatment related, and a skin biopsy revealed only fibrosis.

The biochemical parameters prior to treatment, posttreatment, and prior to initiation of Cycle No. 2 are noted in table 3. After each cycle, a significant decrease in serum calcium, phosphorous, urinary calcium, and urinary hydroxyproline/creatinine ratio was observed. A decrease in osteocalcin was also observed, but the change was not statistically significant. All parameters returned to baseline prior to the second cycle. Parallel with the decrease in total serum calcium, an increase in C-terminal parathyroid hormone was observed. No significant or consistent change in alkaline or acid phosphatase, albumin, 25-(OH) vitamin D_3, 1,25-$(OH)_2$ vitamin D_3, or lactate dehydrogenase levels was observed. The reversibility of the effect on calcium and phosphorus is noted in figure 2, which shows pre- and posttherapy and prior to recycling values for sequential treatments. The statistical results are summarized in table 4. Thus, it would appear that the effect of gallium nitrate on bone lasted < 4 weeks. No patient demonstrated an improvement by bone scan during the treatment period.

DISCUSSION

The observed response pattern highlights the heterogeneity of human prostatic cancer and emphasizes the role of therapies directed toward specific sites. While two patients (10%; 95% confidence limits, 0%–22%) demonstrated regression of soft tissue disease, seven of 23 (30%; 95% confidence limits, 14%–52%) noted improvement in bone pain. A dissociation of clinical response and objective tumor regression was observed in five cases. The toxicity of gallium observed in this study was

TABLE 3.—Change in biochemical parameters following treatment with gallium nitrate

	No. of patients	Baseline		Day 8		Day 28	
		Median	Range	Median	Range	Median	Range
Calcium	23	8.9	8.0–10.4	8.1	6.7–9.2	8.8	7.7–12.4
Phosphorus	23	3.7	1.2–7.5	2.3	1.2–3.6	3.6	0.9–4.3
Alkaline phosphatase	23	368	37–2115	358	39–1478	271	47–2218
Acid phosphatase	21	6.2	0.3–145	3.6	0.3–85	8.9	0.5–156
Lactate dehydrogenase	23	232	158–1308	231	148–1052	225	128–2105
C-terminal parathyroid hormone	20	281	50–1014	502	112–953	294	33–921
25-(OH) vitamin D_3	20	19	8–42	17	3–41	16	3–58
1,25-$(OH)_2$ vitamin D_3	19	39	5–98	39	5–106	36	5–122
Osteocalcin	17	19	4.2–67	12	0.5–24.7	17	4.0–63.8
Urine calcium	17	111	8–470	20	3.9–260	67	10–349
Hydroxyproline/creatinine	19	6.7	0.8–16	3.4	1.4–17	6.4	1.4–49.2

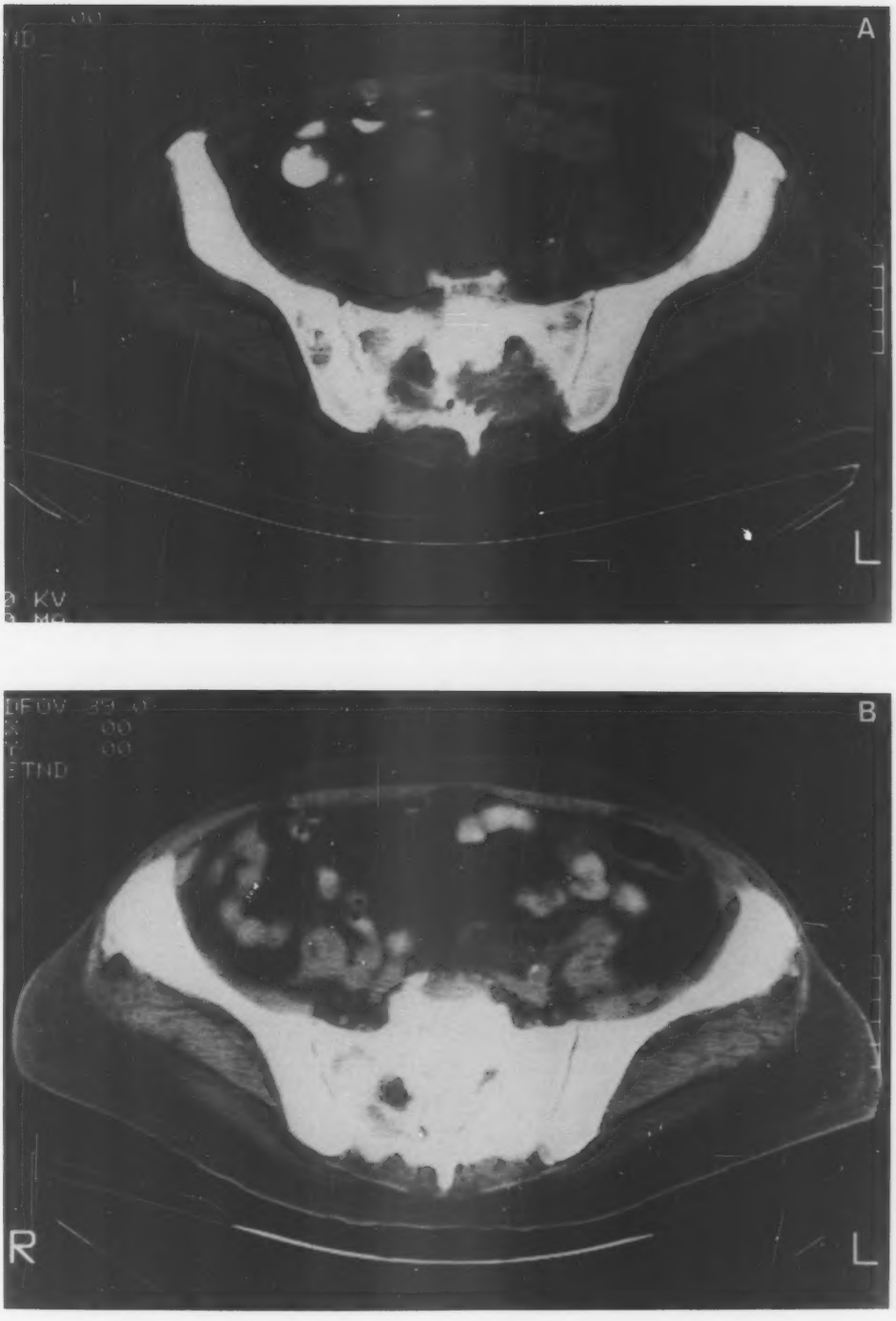

FIGURE 2.—Response in a patient with a presacral mass and contiguous bone destruction. (A) Prior to treatment with gallium nitrate. (B) After 4 cycles of therapy. Note decrease in soft tissue component with calcification of previous lytic area.

TABLE 4.—Statistical comparison of biochemical parameters at baseline, Day 8, and Day 28

	No. of patients	χ^2	DF*	*P* value	Pairwise comparisons†
Calcium	23	32.44	2	≤ 0.001	Days 0 and 28 differ from Day 8
Phosphorus	23	25.2	2	≤ 0.001	Days 0 and 28 differ from Day 8
Alkaline phosphatase	23	1.83	2	0.49	
Acid phosphatase	15	0.23	2	0.73	
Lactate dehydrogenase	23	3.28	2	0.19	
C terminal parathyroid hormone	12	7.3	2	0.03	Days 0 and 28 differ from Day 8
25-OH vitamin D_3	17	4.09	2	0.13	
1,25-$(OH)_2$ vitamin D_3	18	2.53	2	0.28	
Osteocalcin	8	3.43	2	0.18	
Urine calcium	14	9.57	1	0.008	
Hydroxyproline/creatinine	19	5.55	2	0.06	

*Friedman's test (ref 25) $P \leq 0.05$ considered significant.
†Friedman's test (ref 25) $P \leq 0.0167$ considered significant.

similar to those seen in previous investigations (25). Despite the known propensity for prostatic cancer to produce osteoblastic lesions with concomitant hypocalcemia (2,26,27), the incidence of hypocalcemia in the present series was similar to previous studies (25).

Serum calcium, serum phosphorus, urinary hydroxyproline/creatinine ratios, and urinary calcium excretion were consistently lower at the completion of the drug infusion. However, levels had returned to baseline prior to the next cycle. It is therefore possible that any beneficial effect on bone using this dose schedule of gallium nitrate was of too short a duration to produce persistent clinical improvement. In the series by Adami et al (13), using dichloromethylene diphosphonate (an agent that inhibits bone resorption), clinical improvement was observed after 14 days with a parallel decrease in urinary calcium and urinary hydroxyproline/creatinine ratios. No antitumor activity was observed. No other consistent changes in measured biochemical parameters were observed.

In contrast to other tumors, the predominant metastatic pattern produced by prostatic cancer in bone is osteoblastic rather than osteolytic. Normal bone remodeling proceeds via a coupled mechanism of resorption followed by formation (28,29). In prostatic cancer, direct bone formation without resorption has also been observed, which is probably related to a recently identified osteoblast growth factor which stimulates mitogenesis in fetal rat calvarial-derived osteoblasts (30,31) and in osteosarcoma cell lines (32). However, the observation of increased urinary hydroxyproline/creatinine ratios in previous reports (8–11) and in the present series, increased osteoclast activity in the area of tumor (26,27,33), and histomorphometry showing increased resorptive activity in tumor versus nontumor sites (12,34) suggests that despite tumor-induced bone formation, coupling is not lost in all cases.

Prostatic cancer continues to be a disease refractory to therapy. Thus, strategies aimed at inhibition of bone resorption deserve further study. The increased understanding of the interactions of tumor and bone may allow the evaluation of different classes of agents (35,36).

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Pilot Study of Induction Therapy With Cyclophosphamide, Doxorubicin, and Cisplatin (CAP) and Chest Irradiation Prior to Thoracotomy in Initially Inoperable Stage III M_0 Non-Small Cell Lung Cancer[1,2]

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In an attempt to increase the resection rate of patients with non-small cell lung cancer, two cycles of cyclophosphamide, doxorubicin, and cisplatin chemotherapy and chest irradiation (3000 cGy in 10 fractions, split course) were administered to 42 initially inoperable (T_3 or N_2M_0) patients. Three patients were subsequently determined to be ineligible, two because of small cell histology and one because of M_1 disease. Among the 39 eligible patients, 20 (51%) had a regression following the combined-modality therapy. Seventeen of these patients plus two with stable disease had thoracotomy. Complete surgical resection was accomplished in 13 patients (33% of all eligible 39 patients; 68% of all 19 patients who had thoracotomy). There were no statistically significant survival differences between patients not having thoracotomy and those who had thoracotomy (or even those with complete tumor excision). The patients with chemotherapy, chest irradiation, and surgical resection had a low incidence of local in-chest failure (5%), but distant failure was still a major problem. [Cancer Treat Rep 71:895-900, 1987]

Lung cancer will develop in an estimated 149,000 people in the United States in 1986 (1), approximately 80% of whom will have non-small cell histologies (2). Of these 120,000 patients, about 50% will have distantly metastatic disease, 20% will have resectable tumors, and 30% (35,000-40,000 patients) will prove to have disease limited to the primary tumor with or without hilar, mediastinal, or ipsilateral supraclavicular lymph nodes and one or more criteria of unresectability, Stage III M_0 (3). Successful treatment aimed at improving the survival of this group of patients with locally advanced inoperable disease to match that of patients with resectable localized disease would result in a substantial saving of lives.

Lung cancer is staged by the American Joint Committee (3) criteria as Stage III when regional spread is

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present. Limited Stage III M_0 is used to designate tumors confined to one hemithorax which can be encompassed by a single radiotherapy port. The majority of the limited Stage III M_0 tumors are treated with radiation therapy when the cell type is non-small cell lung cancer (NSCLC). With this treatment modality, 5-year survival probability is about 5% (4–6). Most of the deaths following radiation therapy are from distant metastases. However, local failure only is the first site of failure in 13%–23% of patients (7). To improve the survival in the limited Stage III M_0 patients, both local recurrences and distant metastases must be reduced.

The Lung Cancer Study Group (LCSG) designed a protocol (831) for limited Stage III M_0 NSCLC patients to determine whether combined induction chemotherapy and radiation therapy can reduce the tumor mass sufficiently to warrant an attempt at surgical resection, to determine what proportion of such patients have an apparently complete resection, and to assess toxicity from the combined-modality approach. The chemotherapy-radiation therapy used was similar to that reported by Eagan et al (8) and was chosen because of the group's experience with this approach from an earlier protocol.

MATERIALS AND METHODS

Forty-two patients with limited Stage III M_0 histologically confirmed NSCLC were entered in the study during 1983–1984. Eligibility requirements included: serum creatinine $\leq$ 1.5 mg/100 ml; Karnofsky performance score $\geq$ 70; $<$ 10% weight loss in the 3 months preceding entry; cardiopulmonary function sufficient to permit chest irradiation and pneumonectomy; measurable or evaluable disease; and signed informed consent. Contraindications included: superior vena caval obstruction; pleural effusion; uncontrollable congestive heart failure; arrhythmia; myocardial infarction (within preceding 3 months); leukopenia or thrombocytopenia; and history of lung cancer or history of other cancer (excluding nonmelanoma skin cancer and in situ cervical cancer) unless a 5-year disease-free interval existed.

Prior to entry, the following tests were performed: history and physical examination; chest x-ray; routine serum chemistry profile; cbc; radionuclide bone scan or x-ray skeletal survey; liver imaging by radionuclide scan or computerized tomographic (CT) scan; mediastinal CT or tomograms; CT of the brain with contrast medium; forced vital capacity; and bronchoscopy. Prior to the start of each new chemotherapy cycle, patients were re-evaluated by: history and physical examination; chest x-ray; chemistry and hematology groups; and evaluation of indicator disease. Response was assessed by the criteria of Eagan et al (8). Measurable disease criteria included: *complete regression (CR)*—the complete disappearance of all evidence of disease; *partial regression (PR)*—a $\geq$ 50% reduction in tumor size with no new evidence of disease; *disease improvement*—a 25%–49% reduction in tumor size with no new disease; *stable disease*—a $<$ 25% decrease or increase in tumor size with no new disease; and *progressive disease*—a $\geq$ 25% increase in tumor size or any new sites of disease. Evaluable disease criteria included: *disease regression*—definite decrease in tumor size; and *disease progression*—definite increase in tumor size; otherwise, the disease was stable. Any new disease indicated disease progression. All evaluations required two trained investigators.

The treatment schema (fig 1) called for three cycles of chemotherapy and two 5-day sessions of radiation therapy (concomitantly with chemotherapy Cycles 2 and 3) unless disease progression occurred, at which point the patient was taken off study. After that treatment, patients in the CR, PR, or disease regression categories were to have thoracotomy. Patients with lesser regression or who were unresectable at thoracotomy were scheduled to complete the chest irradiation.

Chemotherapy consisted of the iv administration of CAP (cyclophosphamide at 400 mg/m^2, doxorubicin at 40 mg/m^2, and cisplatin at 60 mg/m^2) every 4 weeks for three cycles. During the second and third cycles, concomitant chest radiation at a dose of 300 cGy/day for 5 days (1500 cGy) to the primary tumor and lymph node drainage areas was to be given for a total of 3000 cGy in 10 fractions.

RESULTS

Forty-two study patients were entered between June 16, 1983, and March 28, 1985. One of these patients was

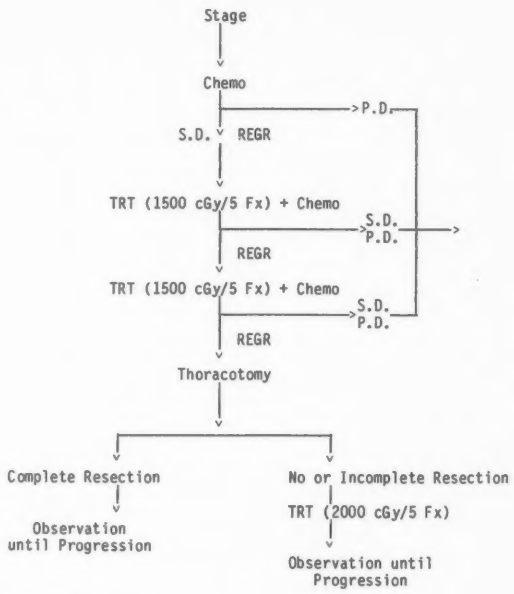

FIGURE 1.—Schema for LCSG Protocol 831.

TABLE 1.—Initial characteristics for the 39 eligible patients in Protocol 831

Characteristic	No. of patients
Sex	
Male	29
Female	10
Tumor status	
Evaluable	18
Measurable	21
Initial cell type	
Squamous cell carcinoma	8
Large cell undifferentiated carcinoma	9
Adenocarcinoma	21
Adenosquamous carcinoma	1
Site of primary tumor	
Right upper lobe	22
Right middle lobe	1
Right lower lobe	3
Left upper lobe	8
Lungula	1
Left lower lobe	3
Other	1
Age (yrs)	
Mean: 59.2	
Range: 44–78	
Median: 60	
SD: 7.7	

excluded as ineligible because of M_1 disease status (scalene node metastases). Two additional patients found to have small cell undifferentiated cancer on review by the Pathology Reference Center were also excluded. This resulted in 39 eligible and evaluable patients. All entry testing was done except: bronchoscopy (one patient), mediastinal CT scan or tomograms (two), and CT scan of the brain with contrast medium (seven).

Among the 39 eligible and evaluable patients, there were 29 males and ten females, with a combined median age of 60 years (range, 44–78). Twenty-one patients had measurable tumors and 18 had evaluable tumors. The primary tumor involved the upper lobes in 30 of 39 patients (77%). The histological diagnoses among eligible patients were: squamous cell carcinoma (eight patients); large cell undifferentiated carcinoma (nine); adenocarcinoma (21); and adenosquamous carcinoma (one). These initial characteristics are shown in table 1. The TN stages of the patients at diagnosis and after induction therapy and the methods of diagnosis are shown in tables 2–4.

TABLE 2.—TNM status at diagnosis and after induction therapy*

	Pretreatment stage				
Posttreatment stage	T_1N_2	T_2N_2	T_3N_1	T_3N_2	Total
T_1N_0	1	1	0	1	3
T_1N_1	0	1	0	0	1
T_1N_2	0	1	0	0	1
T_2N_0	1	3	0	0	4
T_2N_1	0	1	0	0	1
T_2N_2	0	10	0	1	11
T_3N_0	0	0	0	1	1
T_3N_2	0	5	0	2	7
Unknown	4	4	2	0	10
Total	6	26	2	5	39

*Values = No. of patients.

TABLE 3.—Method used to determine T status*

Method	Before treatment	After induction therapy
Bronchoscopy	10	4
CT scan	8	15
Chest x-ray	17	14
Other	2	0
Unknown	2	6
	39	39

*Values = No. of patients.

Response to Induction Chemotherapy and Radiotherapy

Eight of 39 patients (21%) had regression following the first cycle of chemotherapy alone and 20 of 39 (51%) had regression (table 5) during the three-cycle induction period with chest irradiation (two CRs, four PRs, and 14 regressions). Two of eight patients (25%) with squamous histology regressed compared with 18 of 31 patients (58%) with adenocarcinoma, large cell carcinoma, or mixed cell types. Because of small numbers, this apparent difference was not statistically significant.

Surgery

Of the 20 patients eligible for thoracotomy because they were classified as having a tumor regression, 19 actually had surgery. One patient refused surgery, one patient was felt to be inoperable because of persistent esophageal involvement, and one patient was inoperable because of poor cardiopulmonary reserve. In addition, two patients classified as having stable disease had thoracotomy. In summary, 20 of 39 patients (51%) became eligible for surgery and 19 of 39 (49%) actually had surgery. The technical steps for surgical resection were somewhat more difficult because of the effects of radiation therapy, doxorubicin, and advanced disease. There were no surgical deaths and no bronchopleural

TABLE 4.—Method used to determine N status*

Method	Before treatment	After induction therapy
Biopsy	33	4
CT scan	1	14
Routine x-ray	1	12
Other	2	3
Unknown	2	6
	39	39

*Values = No. of patients.

TABLE 5.—Response to induction chemotherapy/radiotherapy

	Evaluable but not measurable tumor		Measurable tumor	
	Surgery	No surgery	Surgery	No surgery
< 3 cycles due to failed induction	0	7	0	2
CR	2	0	0	0
PR	0	0	4	0
Disease regression	9	3	2	0
Stable disease	0	4	2	0
Disease progression	0	1	0	0
Distant metastases*	0	2	0	1

*These patients had local regressions but did not receive surgery because of distant metastases. Values = No. of patients.

fistulae or other complications felt to be uniquely related to the radiation therapy or chemotherapy.

At thoracotomy, one patient was discovered to have liver metastases by palpation through the diaphragm and biopsy and no resection was attempted. Of the remaining 19 patients, six had incomplete resections and 13 had complete resections (table 6). Complete resections were possible in patients with both squamous and nonsquamous histologies. Complete resections were achieved among all categories of tumor response (table 6) with no gross or microscopic tumor left behind. Among the 19 patients in whom resection was attempted, all had some lymph node resection and 14 of the 19 had resection of bronchopulmonary, hilar, subcarinal, and paratracheal lymph nodes.

Survival

The median survival for the 39 eligible patients was 11.0 months, with projected 1- and 2-year survival of 43% and 8%, respectively (fig 2). For the 19 patients undergoing thoracotomy, the median survival was 15.0 months, with 1- and 2-year survival of 60% and 10%, respectively. The best survival times were noted among the 13 patients with complete tumor resection at surgery: median survival time was 15.0 months, with 1- and 2-year survival of 70% and 12%, respectively. However, as can be seen from figure 3, apparently complete surgical resection did not amount to cure.

TABLE 6.—Resectability by cell type and response to induction treatment*

Cell type	Complete resection	Incomplete resection
Squamous cell carcinoma		
CR or PR	1	1
Disease regression	1	0
Stable disease	1	0
Nonsquamous cell carcinoma/mixed cell carcinoma		
CR or PR	3	1
Disease regression	6	3
Stable disease	1	0
Unknown (regression)	0	1
Total	13	6

*Values = No. of patients.

Sites of Treatment Failure

The sites of first treatment failure for the 19 patients who had tumor resection are shown in table 7. As can be noted, only 5% of these failures were intrathoracic (local). The brain was the most common first site of distant metastasis. Only two of the 19 patients with thoracotomy (11%) and a total of five of all 39 patients (13%) had autopsies.

Toxicity

The principal toxic effects from CAP chemotherapy were nausea and vomiting, myelosuppression, and alopecia, as expected. Radiation esophagitis occurred in one-third of irradiated patients. No surgical deaths (deaths within 30 days of surgery) were reported. The median hospital stay was 10 days, the longest being 47 days. In the 31 patients for whom both preinduction and postinduction weights were available, weight increased (although not significantly) during chemotherapy and radiation therapy by an average of 0.4 kg (standard error, 0.7).

DISCUSSION

The 49% thoracotomy rate and 33% complete resection rate in this study are better than the rates from similar studies in the more chemotherapy- and radiotherapy-sensitive small cell lung cancer (9). In a group of NSCLC patients with, perhaps, somewhat less mediastinal disease treated with the same CAP chemotherapy and similar radiation therapy, Skarin et al (10) reported resections in 11 of 12 patients. Trybula et al (11) reported surgical resection in 18 of 27 NSCLC patients after chemotherapy with 5-fluorouracil and cisplatin plus chest radiation therapy.

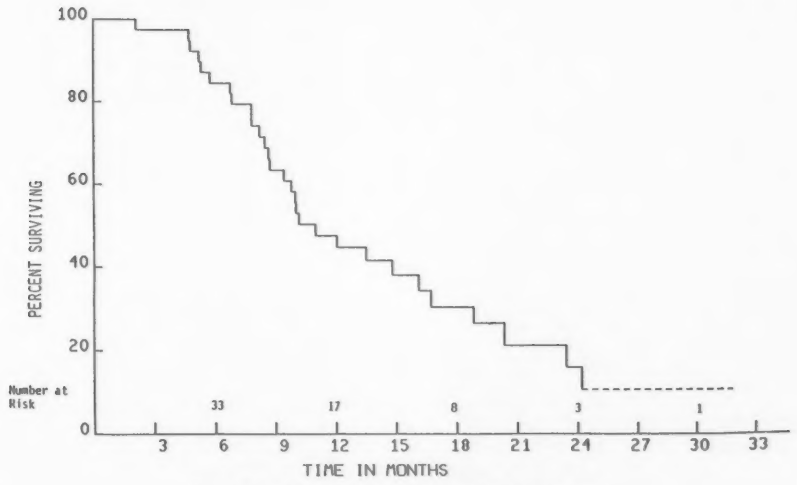

FIGURE 2.—Time to death from entry for all 39 patients.

A question that must be raised is how this combined surgery plus chemotherapy and radiotherapy group compares (from a survival standpoint) to similar patients treated with similar chemotherapy and radiation therapy but without surgery. In a report by Eagan et al (8) on treatment of NSCLC with chest radiation therapy and doxorubicin/cisplatin-based chemotherapy, the median survival for the reported 109 patients was 11.4 months, with 1- and 2-year survival of 48% and 17%, respectively. These figures are somewhat better than those of the current report. However, 13 patients with apparently complete resection are insufficient to permit reliable estimates of survival.

This study showed that surgical resection, complete in most patients, is possible after induction chemotherapy and radiation therapy in about half of NSCLC patients who present with what is traditionally thought of as inoperable Stage III M_0 disease. The study also seems to indicate that there is a definite need for better chemotherapy and radiation therapy to both decrease the bulk and extent of intrathoracic disease and obliterate distant micrometastases.

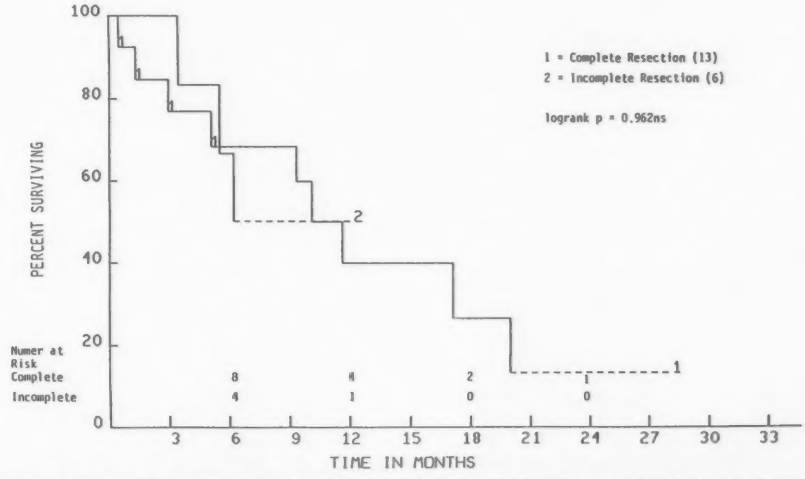

FIGURE 3.—Time to death from surgery for patients receiving a complete resection vs those having an incomplete resection.

TABLE 7.—Sites of first proven recurrence by type of resection for the 19 thoracotomy patients

Type of resection	No. of patients
Complete	
Local only	0
Brain only	4
Other	
Bone*	2
Skin	1
Other*	2
Without disease recurrence	5
Incomplete	
Local only	1
Brain only	0
Liver	1
Without disease recurrence	4

*1 patient had a multiple site of first recurrence (bone and kidney) and is counted in each category. Otherwise, each patient is counted only once.

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Estrogen Receptor-Binding Affinity and Cytotoxic Activity of Three New Estrogen-Nitrosourea Conjugates in Human Breast Cancer Cell Lines In Vitro[1,2]

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The hypothesis that more selective antitumor activity may be achieved by cytotoxic agents which selectively bind to estrogen receptors (ER) in human cancer cells was tested. We have synthesized three nitrosourea derivatives of estradiol or hexestrol, and compared the ER binding affinity and cytotoxic activity of these compounds against ER-positive and -negative breast cancer cell lines in vitro. Specific binding to ER in the cytosol of MCF-7 human breast cancer cells was demonstrated in these conjugates: 17α-CNU > 17β-CNU > HEX-CNU > lomustine (CCNU). The order of cytotoxicity of these derivatives against human breast cancer cells appeared to correlate with their binding affinity to ER. All three estrogen nitrosourea conjugates were more cytotoxic than CCNU, a clinically useful antitumor nitrosourea which does not bind to ER. The contribution of the estrogen moiety to the cytotoxicity of 17α-CNU was demonstrated by the greater activity of the conjugate than that of a combination of estrogen and CCNU. However, cytotoxicity of these compounds against the receptor-positive MCF-7 and receptor-negative Evsa-T human breast cancer cell lines was similar. The latter finding suggested that cytotoxicity of these conjugates may not be mediated through ER. The difference in stability of these nitrosourea conjugates in aqueous buffer may partly explain their differences in cytotoxicity. [Cancer Treat Rep 71:901-906, 1987]

A major deficiency of cancer chemotherapy is a lack of specificity of action; normal tissues may demonstrate the same order of sensitivity as the neoplastic target. The concept of specific delivery of cytotoxic agents to a tumor by linking the agent to a natural substrate, acting as a carrier, has been attempted (1). Though estrogen is itself an effective agent in the treatment of some tumors of hormonal origin, using estrogen as a carrier of alkylating agents has not been a particularly successful approach in producing selective antitumor activity (2,3).

Clinical studies have shown that about 50% of human breast cancers contain estrogen receptor (ER) protein, but only 55%–60% of such ER-positive patients respond to hormonal therapy (4). Accordingly, it was of interest to determine whether the presence of ER, with its specific binding properties, might concentrate cytotoxic agents in such ER-positive tumor cells and thereby achieve more selective antitumor activity.

Certain structural requirements are needed for a compound to bind to ER, eg, availability of the 3- and 17β-hydroxyl groups in estradiol and the 4,4′-hydroxyl groups in the diphenyl nonsteroidal synthetic estrogens (5–8). The failure of previously synthesized estrogen-alkylating agent conjugates, eg, estramustine phosphate and estradiol mustard, to exhibit selective cytotoxicity against breast cancer was attributed in part to their inability to bind to ER and thereby to accumulate preferentially in tumor cells (2,3).

To test the hypothesis that the specific binding property of ER can be utilized to enhance the therapeutic efficacy of an alkylating agent, we have synthesized a series of estrogen-nitrosourea conjugates which retain one or both hydroxyl groups on the estrogen moiety; such groups are essential for the binding of the conjugates to the receptor. In this paper, we report on the binding affinity and the antitumor activity of these agents against ER-positive and -negative human breast cancer cell lines.

MATERIALS AND METHODS

Materials

The following compounds were obtained from the source indicated: estradiol (Sigma); [2,3,6,7-^{3}H(N)]-estradiol (101.7 Ci/mmol, New England Nuclear); charcoal,

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[2] Supported by a grant from the National Cancer Institute of Canada. H.-Y. P. Lam is a Scholar of the Leukemia Society of America.
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Norit A (Sigma); dextran, clinical grade (Sigma); PCS (BDH); ethylenediamine-tetraacetic acid (EDTA, Fisher); tris-hydroxymethylaminomethane (Tris, Sigma); Bacto-trypsin (1:250, Difco Lab); Dulbecco's phosphate-buffered saline (PBS, Gibco); Dulbecco's modified Eagle's medium (Gibco); fetal calf serum (Gibco); and Hanks' balanced salt solution without Ca^{2+} and Mg^{2+} (Gibco).

Synthesis

We synthesized the following three compounds: *N*-(2-chloroethyl)-*N'*-(3,17β-dihydroxyestra-1,3,5(10)-trien-17α-yl)methyl-*N*-nitrosourea (17α-CNU); *N*-(2-chloroethyl)-*N'*-(3-hydroxyestra-1,3,5(10)-trien-17β-yl)-*N*-nitrosourea (17β-CNU); and *N*-(2-chloroethyl)-*N'*-2,3-di(*p*-hydroxyphenyl)-pentanyl-*N*-nitrosourea (HEX-CNU).

Synthesis of 17α-CNU and 17β-CNU and their corresponding amino steroid precursors has been described previously (9).

HEX-CNU was synthesized by condensing 1-amino-2,3-di(*p*-hydroxyphenyl)-pentane (10) with (2-chloroethyl)-nitrosocarbamoyl azide (9). Its structure has been verified by infrared, nuclear magnetic resonance and mass spectrometry (to be published).

Cell Lines and Cultures

MCF-7, an ER-positive human breast cancer cell line, was supplied by Dr. R. Shiu, Department of Physiology. Evsa-T, an ER-negative human breast carcinoma cell line developed by Dr. M. Lippman, National Institutes of Health, Bethesda, MD, was a gift from Dr. Lippman. Unless otherwise stated, all cells were cultured in Dulbecco's modified Eagle's medium (Gibco cat. # 430–1600) supplemented with 10% fetal calf serum, 1 μg/ml of insulin, 3.5 mg/ml of glucose, 100 units/ml of penicillin-G, and 100 μg/ml of streptomycin (growth medium). They were maintained at 37°C in moist 5% CO_2 atmosphere.

Preparation of MCF-7 Tumor Cell Cytosolic Receptor

MCF-7 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% dextran-coated charcoal-stripped fetal calf serum, 1 μg/ml of insulin, 3.5 mg/ml of glucose, 100 units/ml of penicillin-G, and 100 μg/ml of streptomycin. Cell cultures in near-confluent T-150 flasks were rinsed by 10 ml of 0.02% EDTA prepared in Ca^{2+}/Mg^{2+}-free Hanks' balanced salt solution. Cells were harvested by scraping in 10 ml of ice-cold PDG buffer (5 mM sodium phosphate, pH 7.4; 10 mM dithiotreitol; and 10% glycerol). After centrifugation at 600 $\times$ *g*, the cells were resuspended in 0.5 ml of PDG buffer and homogenized in a Dounce homogenizer using the B-pestle until 95% of the cells were disrupted (about 60 strokes) as determined by phase contrast microscopy. The nuclei and cell debris were removed by centrifugation at 800 $\times$ *g* for 10 minutes. The pellet was washed twice with 0.5 ml of PDG buffer, and the supernatant was combined with the 800 $\times$ *g* cell homogenate supernatant. The combined supernatant was then centrifuged at 180,000 $\times$ *g* for 30 minutes at 4°C. The resulting supernatant was diluted with PDG buffer to a protein concentration of 2 mg/ml and used immediately as the cytosol preparation. Protein was determined by the method of Bradford (11) using Bio-Rad reagents (Missisauga, Ontario, Canada) and bovine serum albumin as standard.

Competitive Binding Assay

A modified dextran-coated charcoal adsorption method as described by Katzenellenbogan et al (6) was performed. Various concentrations of drugs (diluted in 1:4 dimethylformamide:PDG buffer) and a final concentration of 10 nM [^{3}H]-E_2 were pipetted into the wells of a 96-well microtiter plate on ice. An aliquot of MCF-7 cytosol was added such that the final volume was 70 μl and the concentration of dimethylformamide was 3%. After incubation at 0°C–4°C for 16 hours, 10 μl of dextran-coated charcoal (5% Norit-A charcoal, 0.5% dextran in phosphate buffer) was added to each well. The plate was vortexed in a shaker at 4°C for one-half hour. The plate was then centrifuged at 800 $\times$ *g* for 15 minutes to precipitate the charcoal. The supernatant from each well (50 μl) was removed and counted for radioactivity in 10 ml of scintillation counting fluid (1:1 PCS/xylene).

The concentration of competitor required to inhibit 50% of the high-affinity [^{3}H]-estradiol binding (IC_{50}) was determined for each competitor. The relative binding affinity (RBA) is defined as the ratio of IC_{50} of unlabeled estradiol to that of the competitor:

$$\text{RBA} = \frac{(IC_{50})\text{estradiol}}{(IC_{50})\text{competitor}} \times 100.$$

Determination of Decomposition of Estrogen-Nitrosourea Conjugates in Aqueous Buffer

The estrogen-nitrosourea conjugates were dissolved in ethanol and then added to PBS buffer, pH 7.4. The final concentration of nitrosourea was 1×10^{-5} M and that of ethanol was 1%. Testosterone was also added as internal standard. This solution was incubated at 37°C or 0°C. At various time points, an aliquot of this solution was taken and extracted with methylene chloride. After drying over anhydrous Na_2SO_4 and concentrating by evaporation of the solvent, a sample was injected into a Beckman high-performance liquid chromatograph Model 110A equipped with a Whatman Partisil PXS 10/25 column and eluted with an isocratic solvent (methylene-isopropanol, 95:5). A Beckman 160 absorbance detector was used to monitor the eluent at 254 mμ. The amount of intact estrogen-nitrosourea is measured from the area under the peak.

Clonogenic Assay

This method is a modification of the procedure described by Goldenberg and Froese (12). Cells grown in

75-cm² culture flasks were trypsinized at half confluency (log phase of growth) with 5 ml of trypsin/EDTA solution. Detached cells were treated with 5 ml of growth medium and pelleted at 600 × *g* for 5 minutes. Cells were resuspended in growth medium supplemented with 20% fetal calf serum at such a concentration so as to yield approximately 5–100 colonies/assay point.

Drugs were added to the cell suspension in ethanol so that the final concentration of ethanol was 1%. Cell suspension (2 ml) was then pipetted into each well of a Linbro 6-well tissue culture plate. Cells were cultured at 37°C in a moist 5% CO_2 chamber for 14 days (MCF-7 cells) or 21 days (Evsa-T). The surviving cells attached to the plate were fixed in methanol and stained with a 1:30 dilution of Giemsa stain (Fisher Chemical Co) for 20 minutes. After two rinses with water, plates were air-dried. Clones of surviving cells (≥ 50) were counted with the aid of an inverted microscope.

RESULTS

Competitive Binding Assay

The structures of E_2, hexestrol, and the three synthetic estrogen-nitrosourea conjugates are shown in figure 1. The competitive binding assay was used to test whether the conjugates bind to ER. The inhibition of [³H]-estradiol binding to ER in MCF-7 cytosol by various competitors is shown in figure 2. The relative binding affinities of 17α-CNU, 17β-CNU, and HEX-CNU were 1.8, 0.41, and 0.14, respectively (the relative binding affinity for E_2 is defined as 100).

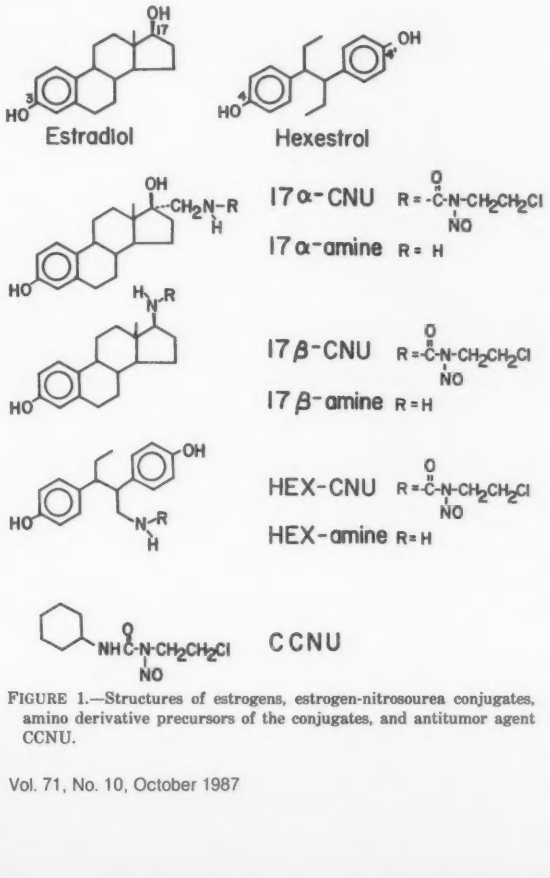

FIGURE 1.—Structures of estrogens, estrogen-nitrosourea conjugates, amino derivative precursors of the conjugates, and antitumor agent CCNU.

In contrast, lomustine (CCNU), which has a cyclohexyl instead of a phenolic estrogen moiety, did not inhibit [³H]-E_2 binding, even at a concentration 10,000 times higher than that of the labeled substrate.

As shown in figure 2, the amino derivatives of estradiol or hexestrol, ie, 17α-amine, 17β-amine, and HEX-amine, which are the precursors of the nitrosourea conjugates, all demonstrated relative binding affinity at least two orders of magnitude lower than those of their corresponding nitrosourea compounds.

Decomposition of Nitrosourea in Aqueous Buffer

Since nitrosoureas are chemically unstable compounds, it is possible that the inhibition of binding of [³H]-E_2 was due to the effects of their decomposition products. We studied the decomposition of these nitrosoureas in 0.1 M phosphate buffer, pH 7.4, by high-performance liquid chromatography. At 37°C, the decomposition of these nitrosoureas followed pseudo-first-order kinetics. The half-lives calculated for the three nitrosoureas were: 17α-CNU, 110 minutes; 17β-CNU, 100 minutes; and HEX-CNU, 60 minutes. In contrast, CCNU, which does not bind to ER, was reported to have a chemical half-life of 53 minutes in the same buffer (13). At 0°C–4°C, no decomposition of these three estrogen-nitrosourea conjugates could be detected over a period of 16 hours.

Cytotoxicity Assay of Estrogen-Nitrosourea Conjugates

The cytotoxicity of these estrogen-nitrosourea conjugates in vitro was determined by the clonogenic assay. The cytotoxicity of the conjugates was expressed by plotting surviving cell fraction on a log scale against drug concentration (figs 3 and 4). D_0 (defined as the dose of drug reducing survival to 1/e, ie, 37% of the initial cell population) was derived from the negative reciprocal of the slope of the linear regression equation.

The D_0 values of the estrogen-nitrosourea conjugates and CCNU are shown in table 1. The cytotoxicity of these compounds was in the following order: 17α-CNU > 17β-CNU > HEX-CNU > CCNU. There appeared to be a correlation between binding affinity of these agents to ER and their cytotoxicity; the most toxic compound, 17α-CNU, also showed the highest ER binding affinity.

To evaluate the contribution of the estrogen moiety to cytotoxicity, we studied the effect of a combination of the precursor amine (also a product after hydrolysis of 17α-CNU) and the nitrosourea CCNU, which does not bind to ER. Table 2 shows the results of this study. The toxicity of these compounds was in this order: 17α-CNU > 17α-amine + CCNU > E_2 + CCNU > CCNU > E_2 > 17α-amine. However, the sensitivity of the ER-positive MCF-7 and ER-negative Evsa-T cells to each drug tested did not show any significant difference, as determined by the Student's t test.

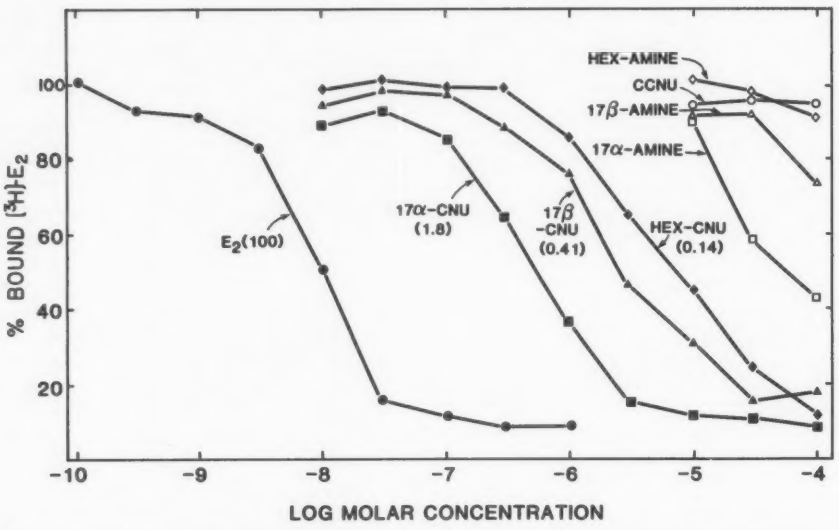

FIGURE 2.—Inhibition of [^{3}H]-estradiol (E_2) binding to MCF-7 cytosol receptors by estrogen-nitrosourea conjugates and precursor amines. Various concentrations of competitors were incubated with 1×10^{-8} M [^{3}H]-E_2 in MCF-7 cytosol for 16 hrs at 4° C as described in the Material and Methods section. Dextran-coated charcoal was added to adsorb unbound ligands. The inhibition of binding of [^{3}H]-E_2 to ER was expressed as the % of radioactivity bound in the absence of the competitors. Nos. in parentheses represent relative binding affinity (*see* text).

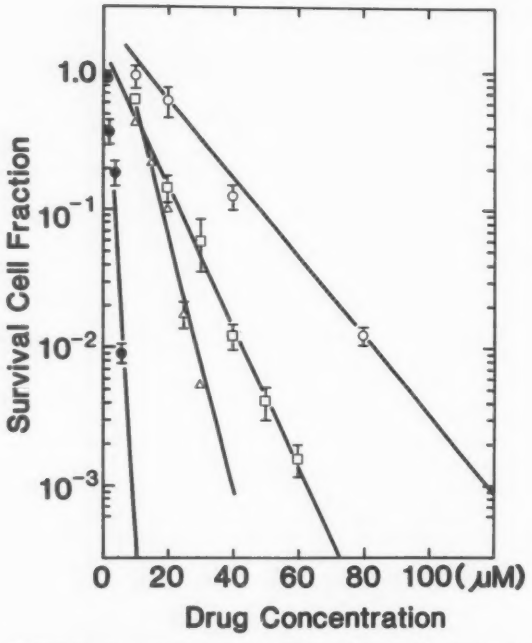

FIGURE 3.—Dose-survival curves of ER-positive MCF-7 cells treated with various concentrations of drugs: CCNU (○); HEX-CNU (□); 17β-CNU (△); and 17α-CNU (●).

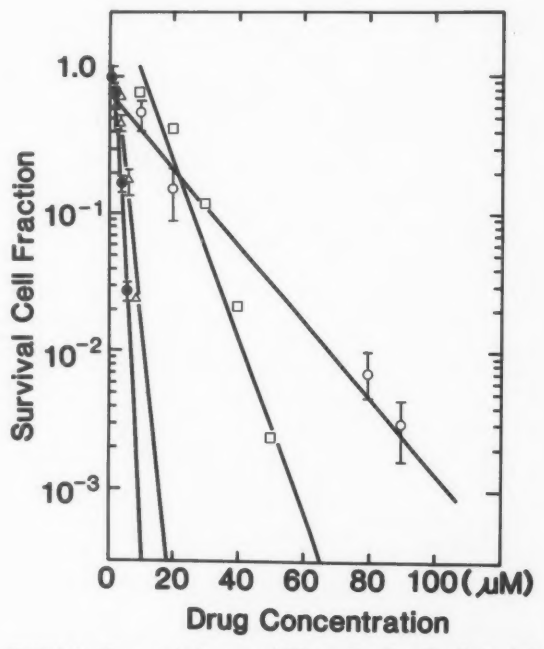

FIGURE 4.—Dose-survival curves of ER-negative Evsa-T cells treated with various concentrations of drugs: CCNU (○); HEX-CNU (□); 17β-CNU (△); and 17α-CNU (●).

TABLE 1.—D_0 values of CCNU and 3 estrogen-nitrosourea conjugates*

Drug	MCF-7 cell line	Evsa-T cell line
17α-CNU	$1.16 \pm 0.02 \times 10^{-6}$ M	$1.18 \pm 0.06 \times 10^{-6}$ M
17β-CNU	$4.47 \pm 0.48 \times 10^{-6}$ M	$3.46 \pm 0.60 \times 10^{-6}$ M
HEX-CNU	$8.29 \pm 0.31 \times 10^{-6}$ M	$6.89 \pm 0.82 \times 10^{-6}$ M
CCNU	$1.66 \pm 0.03 \times 10^{-5}$ M	$1.66 \pm 0.14 \times 10^{-5}$ M

*D_0 is defined as the dose of drug reducing cell survival to 1/e, ie, 37% of the initial cell population. Student's t test showed that for the same drug, there was no significant difference between the D_0 values obtained for the 2 cell lines.

DISCUSSION

To test the hypothesis that ERs in some tumors can specifically accumulate cytotoxic agents, we have synthesized several nitrosoureas conjugated to estrogen derivatives so that the final products retain the necessary structure to permit binding to ER. From the work of several groups of investigators, it has been demonstrated that the 3- and 17β-hydroxyl groups of the estradiol molecule, especially the free 3-hydroxyl group, are essential for the binding of the agent to ER in several tissues from different species (5–8). Our synthetic conjugates all retain at least one of these two free hydroxyl groups (or the 4,4′-hydroxyl groups in the synthetic hexestrol estrogens). All of these compounds were shown to be able to bind to ER. The relatively low binding affinity of these compounds was probably due to the steric hindrance introduced by the nitrosourea moiety. One advantage of using nitrosourea as the cytotoxic agent in these conjugates is that the hydrolysis products of these compounds (13,14), the amino derivatives of estrogens, possess ER binding affinity at least two orders of magnitude lower than that of the corresponding nitrosourea conjugates. Thus, hydrolysis of these compounds in the cell or in the circulation will not yield compounds with higher ER binding affinity such that these hydrolysis products might block the binding of the conjugates to ER (2,3).

Determination of the half-life of these nitrosourea conjugates in aqueous buffers provided evidence that decomposition of these nitrosoureas was minimal in the buffer used in the preparation of ER at 4°C. Therefore, the binding affinities of these agents as determined by the competitive binding assay probably reflect the real binding affinities of these agents rather than that of their decomposition products. However, the possibility that some hydrolytic enzymes present in the cytosol may enhance the decomposition of these nitrosourea conjugates cannot be ruled out. Accordingly, additional studies will be needed to provide definitive proof that the displacement of radioactive E_2 was due to the intact nitrosoureas.

To determine whether the estrogen-nitrosourea conjugates are better anticancer agents than estrogen or nitrosoureas that do not bind to ER, we evaluated the cytotoxic activity of these agents against two human breast cancer cell lines, the ER-positive MCF-7 and the ER-negative Evsa-T. As determined by the dextran-coated charcoal adsorption assay, the ER content of MCF-7 cells was 90–145 fmols/mg of cytosolic protein and that of Evsa-T cells was 0–0.92 fmols/mg of protein. Our results showed that there appeared to be a correlation between cytotoxicity and ER-binding affinity of the nitrosourea conjugates in the following order: 17α-CNU > 17β-CNU > HEX-CNU > CCNU; the higher the binding affinity, the higher the cytotoxicity. However, this spectrum of cytotoxic activity may also be interpreted as an index of chemical stability in aqueous buffer, of the following order: 17α-CNU > 17β-CNU > HEX-CNU > CCNU.

The mechanism of action of nitrosourea compounds is considered to be mediated through generation of carbonium ions and isocyanate species upon decomposition (13,14). The carbonium ions are able to alkylate DNA whereas the isocyanate may carbamoylate target molecules with nucleophilic functional groups, such as proteins. The estrogen-nitrosoureas in this study thus might generate chloroethylcarbonium ions and also an isocyanate from the estrogen moiety. In a study of a series of 2-haloethylnitrosoureas, Wheeler et al (13) have reported that the alkylating activity of a nitrosourea appeared to be the dominant factor in determining antitumor activity of a nitrosourea against murine L1210 leukemia in vivo. The alkylating activity of nitrosoureas appeared to correlate inversely with the chemical half-life of the nitrosourea. On the other hand, the carbamoylating activity of a nitrosourea is a major factor in determining the host toxicity, although it also supplements the alkylating activity in producing a therapeutic response. Our results reported here, with the more stable nitrosourea having the highest cytotoxic activity, seemed to differ from this generalization. However, it should be noted that in our study the cytotoxicity of these nitrosoureas was evaluated against human breast cancer cells in vitro. Under these conditions, it is possible that the slowly released isocyanate derivatives of estrogen might have a more dominant effect in determining cytotoxicity.

The contribution of the estrogen moiety to the cytotoxicity of 17α-CNU was further demonstrated by the clonogenic assay (table 2). The 17α-CNU conjugate was

TABLE 2.—D_0 values of 17α-CNU and combinations of agents*

Drug	MCF-7 cell line	Evsa-T cell line
17α-CNU	$1.16 \pm 0.25 \times 10^{-6}$ M	$1.18 \pm 0.57 \times 10^{-6}$ M
17α-amine + CCNU	$5.85 \pm 0.90 \times 10^{-6}$ M	$7.16 \pm 0.76 \times 10^{-6}$ M
E_2 + CCNU	$1.09 \pm 0.25 \times 10^{-5}$ M	$1.00 \pm 0.06 \times 10^{-5}$ M
CCNU	$1.66 \pm 0.03 \times 10^{-5}$ M	$1.66 \pm 0.14 \times 10^{-5}$ M
E_2	$1.85 \pm 0.54 \times 10^{-5}$ M	$2.10 \pm 0.62 \times 10^{-5}$ M
17α-amine	$2.73 \pm 0.62 \times 10^{-5}$ M	$2.55 \pm 0.27 \times 10^{-5}$ M

*In the determination of cytotoxic activity of drug combinations, equal molar amounts of agents were used at each concentration. Student's t test showed that for the same drug or combination of drugs, the difference between the D_0 values of MCF-7 and Evsa-T cells was not statistically significant.

more potent than CCNU alone or the combination of CCNU with either 17α-amine or E_2. Thus, there appears to be an advantage of administering the conjugate compared to the combination of cytotoxic drug and hormone. Similar cytotoxicity of these conjugates or drug combinations against the ER-positive MCF-7 and the ER-negative Evsa-T cells suggested that the cytotoxicity might not be ER-mediated. Interestingly, a recent report by Wei et al (15) on the cytotoxicity of the nitrosourea and nitrosocarbamate conjugates of the antiestrogen tamoxifen showed that these ER-binding conjugates are more cytotoxic against ER-positive MCF-7 than ER-negative MD-MBA-231 cells. However, as these authors suggested, this selective cytotoxicity might be due to the antiestrogenic activity of their hydrolysis product, bis-desmethyltamoxifen, rather than a direct receptor-mediated, DNA-directed cytotoxic action of TAM-NU itself. We have also studied the potential of 17α-CNU and HEX-CNU as antiestrogens. In the rat system, these two agents showed no antagonistic effect against the uterotrophic effect induced by estradiol, even at a concentration 10,000-fold higher; at high concentrations, these agents were shown to be weak estrogens.[6]

In conclusion, we have shown that conjugation of a cytotoxic nitrosourea moiety to an estrogen derivative enhances the cytotoxicity of both the estrogen derivative and the nitrosourea moieties. This higher cytotoxicity is probably due to greater stability of the conjugates in aqueous media and not due to specific interactions with ER. However, the possibility that the unique structures of these conjugates may direct the cytotoxic nitrosourea moiety to target molecules other than ER cannot be excluded by this study.

[6] Manuscript submitted for publication.

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Actual Versus Ideal Weight in the Calculation of Surface Area: Effects on Dose of 11 Chemotherapy Agents[1,2]

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This study of 2382 breast, 182 rectal, 817 colon, and 351 lung cancer patients treated with combination chemotherapy on eight phase III Eastern Cooperative Oncology Group protocols indicates that 69% would receive a higher dose of at least one drug if surface area were calculated from actual weight rather than from the minimum of actual and ideal weight. Forty-eight percent of the patients would have at least a 10% increase in drug dose based on actual weight and only 8% would have at least a 25% increase in drug dose based on actual weight. Only on the premenopausal adjuvant breast cancer protocol and among women on the rectal adjuvant study do the differences in dose based on actual rather than ideal weight increase significantly with age. On the postmenopausal adjuvant breast study and on the lung cancer study, the differences in dose decrease significantly with age. For all age decades and both sexes within each protocol, the mean differences between dose based on actual and dose based on ideal weights were on the same order as the rounding factors for the 11 drugs studied. From the literature on the effect of doses of common chemotherapies on leukopenia, it appears that the percent of hematologic toxicity would not be raised to unacceptable levels by using actual weight to set doses. [Cancer Treat Rep 71:907-911, 1987]

Most chemotherapy doses are computed on the basis of surface area. Since 1977, the Eastern Cooperative Oncology Group (ECOG) has used the lesser of actual and ideal weight in calculating surface area for all breast cancer protocols. The main purpose of this was to avoid overdosing obese patients. Although ideal weights have been used to set doses in some other cooperative oncology groups and in some other tumors, this practice is by no means universal. In addition, there are many versions of ideal weight charts. If the ideal weight calculation could be omitted from dose calculations, then some time could be saved and protocol doses might be more consistent between studies done by different investigators.

There are three reasons to suspect that the use of actual instead of ideal weight in setting chemotherapy doses might not make much difference in patient toxicity. First, it may be that few cancer patients are very overweight. This would be a reasonable assumption in some cancers commonly associated with prediagnosis weight loss [eg, pancreatic and gastric (1)], but might make less sense in cancers which may be associated with overweight or fat intake [eg, breast and colon (2)]. Second, it may be that the rounding necessitated in delivering both oral and injectable chemotherapies is of the same order of magnitude as any dose differences due to using actual instead of ideal weight. Third, it may be that the dose-toxicity curve for most common chemotherapies is not steep enough for the exact calculation of surface area to make a biological difference in dosing. The ideal way of testing these three suspicions would be a randomized trial of doses using actual weight and doses using the lesser of ideal and actual weight, but this would be a very expensive undertaking which might have to be done for each drug. Second best to a randomized trial would be a detailed pharmacokinetic

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study of several doses both of single drugs and combination regimens to see if blood levels correlate better with surface area based on actual weight. Some of this information might already have been collected by other investigators, but it does not appear to be readily accessible from published studies. In the absence of such data, we decided to use a retrospective analysis to test the first two possibilities.

MATERIALS AND METHODS

The patients analyzed in this study were entered on five concurrently accruing breast cancer protocols (EST-5181 for premenopausal stage II adjuvant patients; EST-4181 for postmenopausal stage II adjuvant patients; EST-2181 for metastatic patients without prior therapeutic chemotherapy; and EST-6181 and EST-7181, both for postmenopausal metastatic patients with prior chemotherapy), on a colon adjuvant study (EST-2276), on the two chemotherapy arms of a three-arm adjuvant rectal carcinoma protocol (EST-4276), and on a small cell lung cancer protocol (EST-1580). None of the nonbreast studies specified that ideal weights were to be used in surface area calculations.

We recalculated only the initial doses of induction therapy since in all protocols later doses were to be based on toxicity as well as surface area. The rounding factors for the 11 drugs with dosage based on surface area are shown in table 1 (only tamoxifen and halotestin were omitted because their doses were not based on surface area). All doses calculated for this study are rounded up, since all five breast cancer protocols specified that doses should be rounded up. For example, if a patient's surface area was 1.5 m^2, then the calculated dose of mitolactol to be given would be 225 mg since $225 > 202.5 = 135 \times 1.5$. The formula (3) used to calculate surface area (m^2) from height (cm) and weight (kg) was $SA = .0235\ H^{.42246}\ W^{.51456}$.

Ideal weight was determined solely from height and sex (see dashed lines in figs 1 and 2). The original source

TABLE 1.—Results by protocols, drug, sex, and age

EST protocol No.	Drug*	Single dose/(m^2)	Rounding factor	Mean difference (mg)†						*P* value for age trend	% of patients receiving—	
				20–29 yrs	30–39 yrs	40–49 yrs	50–59 yrs	60–69 yrs	70–79 yrs		≥ 10% more drug	≥ 25% more drug
5181	C	100	25	6	14	15	20			0.02	45	10
	M, P	40	5	2	6	6	8			0.02	35	8
	F	600	25	33	85	82	124			0.01	34	7
	T^s	12	1	1	1	2	3			0.002	32	7
	A	45	5	3	5	6	9			0.02	33	7
	V_b	4.5	1	0	1	1	1			0.02	43	5
4181	C	100	25			19	18	16	15	0.04	53	12
	M, P	40	5			8	7	7	7	0.12	41	7
	F	600	25			116	110	101	101	0.08	46	8
2181	D	135	25		19	18	22	19	21	0.96	44	5
	A	45	5		6	6	7	7	7	0.60	37	7
7181	D	135	25		19	30	25	20	25	0.88	50	9
	A	45	5		8	7	7	5	9	0.72	42	7
	Mt	9	1		1	3	2	2	2	0.84	53	16
6181	D	135	25			27	20	23	23	0.98	49	7
	A	45	5			2	2	2	1	0.72	39	6
	T^s	12	1			2	2	2	1	0.30	35	8
	V_b	4.5	I			1	1	1	0	0.34	49	3
2276 (males and females)	F	325	25	16	20	30	35	29	28	0.50	20	2
	F	375	25	22	24	37	41	35	33	0.62	22	1
	Mc	130	10	8	9	12	15	12	11	0.60	20	2
4276 (females)	F	325	25			15	30	36	71	0.02	21	2
	F	375	25			15	27	39	79	0.02	21	2
	Mc	130	10			6	12	14	29	0.02	21	2
4276 (males)	F	325	25			17	30	29	14	0.84	13	2
	F	375	25			19	37	39	19	0.74	20	1
	Mc	130	10			7	12	12	6	0.84	13	2
1580 (males and females)	C	700	20			132	86	72	69	0.002	27	1
	M	15	2.5			3	2	2	2	0.004	15	2
	L	70	10			13	9	7	8	0.006	21	2

*C = cyclophosphamide; M = methotrexate; P = prednisone; F = 5-fluorouracil; T^s = thiotepa; A = doxorubicin; V_b = vinblastine; D = mitolactol; Mt = mitoxantrone; Mc = semustine; and L = lomustine.

†Mean difference between dose based on actual weight and dose based on lesser of actual and ideal weight by age.

of the ideal weights is unknown, but its values were specified to apply to people 25–29 years old and are similar to those of the 1983 Metropolitan Life Insurance Company (MLI) study (4), never differing by > 1 kg for females or > 3.5 kg for males. (For both males and females the protocol weights are lighter than the MLIs for short heights and heavier for tall heights.) It may be that the protocol ideal weight chart came from another insurance company or from the MLI chart of a different year.

Confidence intervals for percents used the exact binomial calculation (5). Box plots (6) were used for display purposes. In these diagrams the lower edge of the box represents the 25th percentile, the middle line in the box represents the 50th percentile, the upper edge of the box represents the 75th percentile, and the vertical lines extending from the box represent the smallest and largest observation. The width of the box is proportional to the square root of the sample size; hence, wider boxes indicate less statistical variation and should be viewed as being more precise. The test of increasing percents with age used a test for trend based on the Wilcoxon statistic (7). The test of increasing dose with age used linear regression (8). All tests were two-sided.

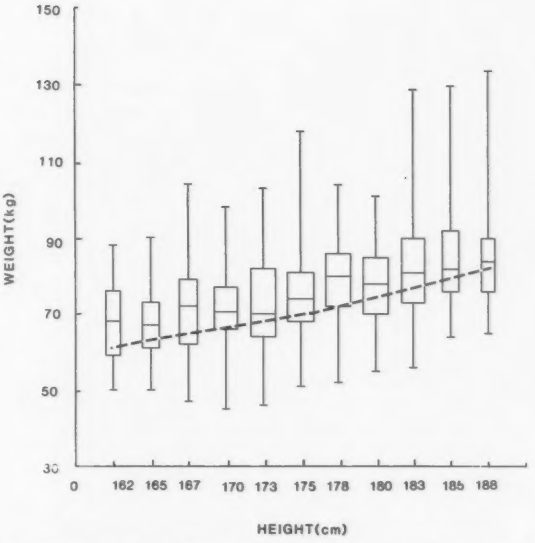

FIGURE 2.—Actual weight by height for 336 males; dashed line = ideal weight.

RESULTS

The total number of patients entered in the eight protocols as of July 1986 is 3947, of whom 215 were excluded from this analysis because the necessary onstudy forms (with weight and height data) had not yet been received, leaving 3732 patients (1307 with adjuvant breast cancer, 1075 with metastatic breast cancer, 817 with colon cancer, 182 with rectal cancer, and 351 with lung cancer). Very few patients were < 30 years old (42 patients) or > 79 years old (11 patients). As expected, weight increases with age. For example, at each height, the median weight of postmenopausal adjuvant breast patients is larger than the median weight of premenopausal adjuvant breast patients. However, the median values never differ by > 7 kg, and there is a large overlap between the 25th and 75th percentiles. For a given height, women on the colon, rectal, and lung protocols tended to be lighter than women on the breast protocols, but the difference in median weights by cancer site was ≤ 10 kg for each height group. Thirty-seven percent of the lung and rectal patients had reported a weight loss in the 6 months prior to study entry, and 28% of the colon patients had reported a weight loss in the 2 months before study entry. Similar data are not available for the breast patients, but it seems reasonable to assume that some of the weight difference by site is due to recent weight loss of the lung and gastrointestinal patients. We also compared average weights within age, height, and sex groups to national averages (9) for the years 1971–1974; in almost all cases the average among cancer patients was < 2 kg greater than the national averages.

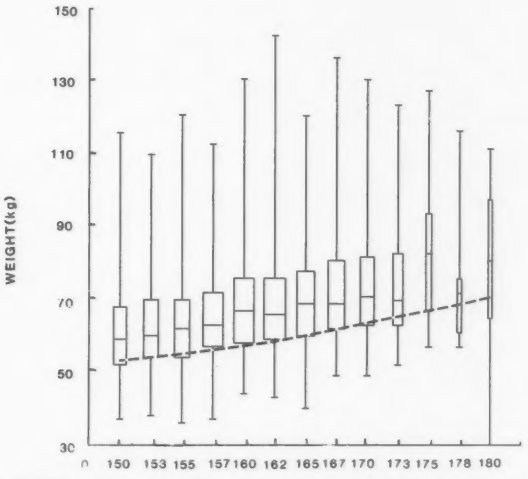

FIGURE 1.—Actual weight by height for 2579 females; dashed line = ideal weight.

The ideal weights are considerably lighter than either the national averages or the average cancer patient's weight. Figure 1 shows actual and ideal weights by height for all 2975 females; figure 2 does the same for all 757 males. Between 62% and 83% of the female patients on each protocol had an actual weight greater than their ideal weight and the median difference between actual and ideal weight ranged from 7.3 to 14.5 kg (12.4–14.5 kg for the breast protocols).

The mean differences of dose calculated from actual weight minus dose calculated from the lesser of ideal

and actual weight are shown in table 1 (doses calculated with rounding factors). The numbers in this table are a function only of the rounding factor and distribution of patient heights, weights, and ages, so drugs with the same rounding factor (eg, methotrexate and prednisone in EST-5181 and EST-4181) have the same outcomes. Since the results in males and females on EST-2276 and EST-1580 are very similar they were combined in the table. On EST-5181, the upward trend with age was significant for all seven drugs. Among women (but not men) on EST-4276, the upward trend with age was significant for both drugs. On EST-4181, the dose difference for cyclophosphamide dropped significantly with increasing age. Among both women and men on EST-1580 the drop in all three drugs was significant. The mean differences in dose were on the same order as the rounding factors in most age-sex-protocol subsets. The only mean differences that were as much as double the rounding factors in most subgroups were 5-fluorouracil on EST-4181 and EST-5181, mitoxantrone on EST-7181, and cyclophosphamide on EST-1580.

Table 1 also shows the percent of patients who would receive at least 10% and 25% more drug using actual weights. Among females, between 13% and 53% of the patients would get at least 10% more drug, and between 2% and 16% would get at least 25% more drug. Among males, between 13% and 32% of the patients would get at least 10% more drug and 1%–2% would get at least 25% more drug.

DISCUSSION

Other studies have determined that blood levels of some nononcology drugs [eg, theophylline (10) and digoxin (11)] correlate more closely with doses based on ideal weight rather than on actual weight. In analogy with drug doses based on ideal instead of actual weight, some chemotherapy groups use ideal weight in the calculation of surface area. Since the use of ideal weight in this calculation is not universal, it is of interest to know how much difference it would make in the initial doses of various types of chemotherapy drugs.

In this retrospective study of 3732 patients entered on eight phase III combination chemotherapy protocols, 11 drugs had doses based on surface area (cyclophosphamide, methotrexate, 5-fluorouracil, doxorubicin, thiotepa, vinblastine, mitolactol, mitoxantrone, lomustine, semustine, and prednisone). Our conclusions are restricted to patients aged 30–79 since very few patients in this analysis had ages outside this range. Seventy-five percent of the patients weighed more than the ideal weights on a chart which was used for calculating doses in the five breast cancer studies. Because both oral and iv preparations required rounding of doses, 8% of these "overweight" patients would receive the same drug doses whether surface areas were calculated from ideal weight or from actual weight. Hence, 69% of all patients would receive more of at least one drug if surface area was based on actual weight. However, most of these patients would have a dose increase $< 25\%$. Only 8% of the patients would have an initial dose increase $\geq 25\%$ if doses were based on actual weight rather than the lesser of actual and ideal. Only two drugs were associated with a 25% increase in $\geq 10\%$ of the patients: mitoxantrone given iv at 9 mg/m^2 and cyclophosphamide given orally at 100 mg/m^2. From single-agent studies (12,13) it seems unlikely that a dose increase $< 25\%$ would result in noticeably more life-threatening toxicity. Also, the mean difference between dose based on ideal weight and dose based on actual weight was seldom much bigger than the difference between doses rounded up and doses rounded down. Since the rounding up of doses has not been reported to be associated with excess toxicity, there is reason to think that the use of actual weight in setting doses would not affect the average patient much.

Since weight increases with age, we expected a priori that using ideal weight for surface area calculations would make a bigger difference at older ages. This seems to be the case only on two of the eight protocols: the premenopausal adjuvant breast protocol and among the women on the rectal adjuvant protocol. Using ideal weight made a bigger difference at younger ages on the postmenopausal adjuvant breast protocol and among both women and men on the lung cancer protocol. On the colon, rectal, and lung studies almost as many men as women had actual weights which exceeded ideal weights, and the difference this would make in initial doses is about the same for the two sexes. However, since there were only 757 men in this study, our conclusions about sex differences in dose are necessarily tentative.

In the absence of pharmacokinetic correlation studies, we suggest that actual weight be used in setting initial chemotherapy doses in most phase III studies (all except those using very high doses, such as bone marrow transplant studies) and in most patients (all except those who are clinically obese in this setting, about 30% overweight). This policy would result in more consistent doses being given to most patients in different institutions and on different protocols. Although it is impossible to estimate the dose toxicity curves from this sort of retrospective study, we do not expect that such a policy would increase life-threatening toxicity.

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Effectiveness of Scalp Cooling in Reducing Alopecia Caused by Epirubicin Treatment of Advanced Breast Cancer[1]

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The value of scalp cooling in the prevention of alopecia was investigated in 32 patients with advanced breast cancer who were given a mean of four courses of 40–80 mg/m² of epirubicin. None of the 15 patients free from liver metastases who received scalp cooling required a wig, whereas four of eight similar patients who did not receive scalp cooling did require a wig. Abnormalities of aspartate transaminase and alkaline phosphatase pretreatment were predictive for reduced efficacy of scalp cooling, but not a contraindication to its use. [Cancer Treat Rep 71:913-914, 1987]

Epirubicin is a new analogue of doxorubicin, differing in the epimerization of the 4′-OH group. This small change results in a significant modification of the metabolic pathway with glucuronidation of the patent compound epirubicin and the metabolite epirubicinol occurring. These glucuronides are found in the plasma and urine in significant quantities, unlike in doxorubicin metabolism. The total plasma clearance of epirubicin is significantly higher than doxorubicin and it is primarily excreted by the liver with a terminal half-life of 30–40 hours in contrast to 40–70 hours for doxorubicin. These differences in metabolism have been presumed to be the reason for the reduced acute toxicity and cardiotoxicity of this drug in phase II studies when compared to doxorubicin (1,2). Epirubicin has a similar spectrum of antineoplastic activity to doxorubicin with established activity in breast cancer. The principal acute side effects are also similar and include nausea, vomiting, myelosuppression, and alopecia.

During a study designed to assess the efficacy and toxicity of this drug when used as a single agent in advanced breast cancer in a routine clinical setting, we evaluated the role of scalp cooling in preventing alopecia. This technique has been shown to be of value when using doxorubicin (3,4), as long as the liver function is not compromised and the excretion of the drug prolonged.

All patients were offered the use of the Howard-Stenner cap (5), which is a double-layered bathing cap filled with cryogel. This was kept overnight in a freezer at −18°C prior to use. The cap was applied over a cotton balaclava, covering wet hair for 15–20 minutes prior to the bolus injection of epirubicin. It remained in place for 45 minutes after the injection. A total of 32 patients, 24–73 years of age, were assessable for alopecia using the World Health Organization (WHO) criteria (6). They had received two or more courses of epirubicin given at 3 weekly intervals. We were also evaluating whether epirubicin has an improved therapeutic ratio compared to doxorubicin. Therefore, a low starting dose of 40 mg/m² was used escalating to 60 mg/m² after two courses where no response was seen, and again to 80 mg/m² if disease was static at this level. The mean number of courses received by each patient was four. Seventy-seven courses of 40 mg/m², 41 courses of 60 mg/m², and eight courses of 80 mg/m² were administered.

RESULTS (fig 1)

A total of 22 patients accepted scalp cooling. Fifteen of these were free from liver metastases and received an average of four courses each. None of these patients required a wig. Thirteen of the 15 had either minimal or no hair loss. Ten patients in this group had received one or more courses at the higher dose of 60 mg/m² and two received six of the eight courses of 80 mg/m² that were administered.

Seven patients with liver metastases, none of whom had a raised bilirubin, received scalp cooling. Four developed WHO grade 2 or grade 3 alopecia. All those with a raised aspartate transaminase and alkaline phos-

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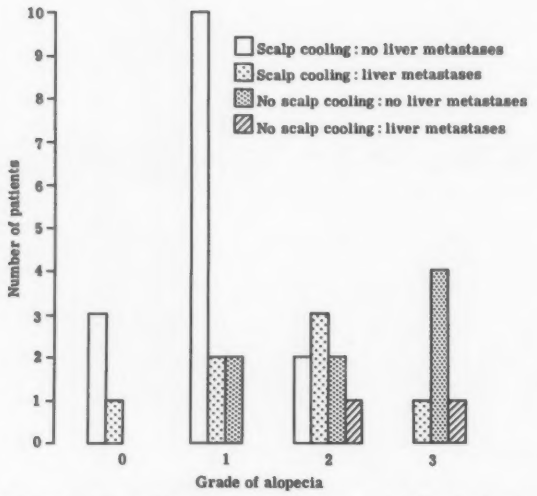

FIGURE 1.—The influence of liver metastases on the efficacy of scalp cooling in reducing alopecia caused by epirubicin chemotherapy for advanced breast cancer.

phatase prior to treatment developed grade 2 or grade 3 alopecia.

Scalp cooling may be an uncomfortable procedure and all patients were informed of this prior to starting chemotherapy. As a result, ten patients did not want scalp cooling. Eight were free from liver metastases. Three of these received epirubicin at 40 mg/m^2 only and five also had one or more courses of 60 mg/m^2. Four of the five patients (who received an average of five courses each) required a wig (WHO grade 3 alopecia).

Only three patients in this study had received previous chemotherapy. This had not caused alopecia. The two patients of this group receiving scalp cooling scored grades 0 and 1 on the WHO scale after two and six courses, respectively. The other patient, not treated with scalp cooling, had developed grade 2 alopecia after two courses.

DISCUSSION

The acute toxic effects of epirubicin, nausea, vomiting, and myelosuppression, have been reported to be less severe than those of doxorubicin. The pharmacokinetics of epirubicin are similar to those of doxorubicin and would suggest that scalp cooling might be effective in preventing alopecia. The reported level of complete alopecia when using epirubicin without scalp cooling varies from 25%–100% (7).

None of the patients with normal liver function required a wig when the epirubicin was given with scalp cooling. When it was not used, 50% of the patients did require a wig. None of the patients studied had a raised bilirubin, but liver enzyme abnormalities probably reduced the efficacy of scalp cooling without rendering it completely ineffective (fig 1).

Two patients stopped scalp cooling after five and six courses, respectively, because of discomfort experienced during this treatment.

Epirubicin has recently been developed to replace doxorubicin in the palliation of advanced breast cancer on the basis of reduced toxicity. Alopecia is one of the most distressing side effects of anthracycline therapy for this group of patients. This simple technique of scalp cooling appears to be as useful in preventing alopecia induced by epirubicin as it is for doxorubicin. This will encourage the use of this drug with its reported advantages over doxorubicin in terms of reduced gastrointestinal toxicity and myelosuppression.

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High-Dose Intensity, Circadian-Timed Doxorubicin and Cisplatin Adjuvant Chemotherapy for Bladder Cancer[1,2]

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Thirteen patients with transitional cell carcinoma of the bladder received monthly circadian-timed doxorubicin and cisplatin chemotherapy immediately after radical cystectomy. In five patients cancer had spread through the serosa of the bladder wall and/or into the perivesical fat (stage C). In eight patients cancer had spread to other pelvic tissues and pelvic lymph nodes (stage D_1). Ten of these 13 patients showed no recurrence of disease after a median follow-up period of 3.5 years (range, 1 to > 5.5); these patients received no chemotherapy for a median duration of 3 years (range, 2–5). Two of the three patients who ultimately failed had local tumor recurrence which occurred much later than is the usual case (40 and 52 months). This circadian-timed two-drug regimen, given in full doses for nine courses as adjuvant treatment, delays and may prevent local and distant recurrence of stage C and D_1 bladder cancer. [Cancer Treat Rep 71:915–919, 1987]

Bladder cancer will kill > 10,000 people in the United States in 1987 (1). Most patients with noninvasive transitional cell carcinoma of the urinary bladder (TCCB) survive for many years, but patients with poorly differentiated, deeply invasive bladder cancers usually die within 2 years of diagnosis (2–5). Neither aggressive surgery (6,7) nor radiation therapy (8,9) has resulted in 5-year survival rates > 35% for patients with cancer infiltrating through the bladder wall into the perivesical fat (stage C) or > 10%–15% for patients with cancer extending to other pelvic organs and regional lymph nodes (stage D_1). Patients with disease that has metastasized beyond the pelvis (stage D_2) survive only weeks to months. Most recent investigations have been directed toward finding effective cytotoxic drugs and drug combinations (10,11). Because a population at high risk for disease recurrence and dissemination can be very reliably defined, the most effective drug combinations for treatment of metastatic TCCB are now being tested in the adjuvant setting, in an attempt to delay or prevent recurrence and spread of these cancers.

To date, cisplatin is the most effective drug used for treating widely metastasized TCCB (11–17). Although it regularly produces objective response rates of 30%–45%, complete responses have rarely been reported (14,16). The relatively short duration (5 months) of cisplatin-induced responses has also been discouraging. Cisplatin combined with drugs such as methotrexate, 5-fluorouracil, doxorubicin, cyclophosphamide, and vinblastine usually produces overall objective response rates higher than those obtained by cisplatin alone. Our review of studies using combination drug regimens revealed a trend toward longer response durations and a higher proportion of complete responses when combinations of drugs were used than when single agents were used (18). Most aggressive multidrug cisplatin-containing regimens (cisplatin, methotrexate, and vinblastine or methotrexate, vincristine, doxorubicin, and cisplatin) (19–22) may produce complete responses in 23%–40% of patients. Pathologically confirmed complete remissions or partial remissions with minimal remaining disease, which can be completely resected, have a median duration > 16 months (21).

We have previously reported that doxorubicin and

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cisplatin combination chemotherapy results in complete remission in a quarter of the patients with widely metastasized or unresectable TCCB (18). This combination of drugs was given according to a circadian-timed schedule that stipulated drug sequence, the interval between administration of drugs, and the time of day drugs were administered. In 1980, because of these earlier results and because of the uniformly dismal prognosis for patients whose bladder cancer had spread outside of the bladder into nodes or contiguous tissues at the time of complete resection of all gross disease, this combination chemotherapy was advanced to the adjuvant setting. Nine monthly circadian-timed doxorubicin and cisplatin treatments were offered to all patients whose disease was completely resected and who were proven by pathological examination to have stage C or stage D_1 TCCB.

Maximum dose intensity has been shown by Hryniuk and Bush (23) and by Goodyear et al (24) to be extremely important for induction of optimal tumor response and the subsequent survival of patients with ovarian cancer. This dose intensity is especially important for drug combinations containing cisplatin. The circadian timing of doxorubicin and cisplatin administration is critical to the maximization of dose intensity (25). Therefore, a circadian-timed treatment schedule, drug sequence, and interval between administration of the drugs were stipulated in this adjuvant chemotherapy study.

MATERIALS AND METHODS

Therapeutic Plan for Patients After Resection of Locally Metastasized Disease

Between August 1980 and November 1984, five patients with stage C TCCB and eight with stage D_1 TCCB were treated with doxorubicin and cisplatin every month, for a planned total of 9 months. Eight of these 13 consecutively diagnosed patients were men and five were women. The median age was 65 years (range, 53–72). Table 1 shows patient characteristics and the extent of their disease.

Only one of the 13 patients had received prior local radiation therapy. Eight patients received the full planned regimen. Of the five patients who did not complete the treatment, three received at least six cycles of therapy, and two refused additional therapy after the fourth cycle. All 13 patients were evaluated for this study.

Patients were staged surgically. Radical cystectomy in females included the resection of uterus, tubes, ovaries, anterior vaginal wall, and urethra. In males it included removal of prostate, seminal vesicles, and perivesicle tissue. All patients had bilateral en bloc pelvic lymphadenectomy and extensive para-aortic node sampling together with bladder resection. Chemotherapy was begun within 1 month of surgery. All patients were treated in the University of Minnesota General Clinical Research Center.

Doxorubicin (60 mg/m^2) was given as a 30-minute iv infusion at either 6 AM or 6 PM, and this was followed 12 hours later by a 30-minute infusion of cisplatin (60 mg/m^2). Hydration was begun immediately after the 30-minute doxorubicin infusion. Normal saline with 20 mEq/L of potassium chloride was infused at a rate of 200 ml/hour for 7 hours; the rate was then increased to 350 ml/hour 5 hours prior to the cisplatin infusion and was maintained at a rate of 350 ml/hour for 4 hours after cisplatin administration. The average hourly urine output during the 9-hour period approached 200 ml/hour. No diuretics were given. A total of nine cycles of this chemotherapy were planned.

Tumor response and/or recurrence evaluations were performed on a regular schedule. History and physical examination, chest x-ray, bone scan, and abdominal and pelvic computerized axial tomographic scan were rou-

TABLE 1.—Patient characteristics and extent of TCCB at cystectomy/lymphadenectomy

Patient No.	Age (yrs)	Sex	Stage	Grade	No. of pelvic lymph nodes		Estimated total tumor mass (g)
					Removed	Infiltrated	
1	64	M	D_1	4	20	5	50
2	55	M	D_1	3	36	10	30
3	59	M	D_1	3	26	3	25
4	66	F	C	4	30	0	20
5	67	M	C	4	49	0	15
6	66	F	D_1	3	12	1	20
7	70	M	C	4	11	0	20
8	67	F	C	3	29	0	100
9	63	M	C	3	30	0	20
10	54	F	D_1	3	34	5	20
11	58	M	D_1	4	27	0*	15
12	73	M	D_1	4	45	1†	50
13	65	F	D_1	4	33	1†	120

*Infiltration of neighbor organs.
†Microscopic infiltration only.

TABLE 2.—Patient status

Patient No.	Follow-up from cystectomy (mos)	Circadian treatment schedule*	No. of cycles completed	Status†
1	17	A	4	Dead‡
2	35	B	9	NED
3	38	B	9	DOC
4	56	B	9	NED
5	46	A	9	NED
6	50	B	9	NED
7	67	A	9	NED
8	42	A	6	NED
9	23	B	6	Dead
10	56	A	9	AWD
11	46	B	9	Dead
12	57	A	7	NED
13	13	B	4	DOC

*Schedule A = treatments started at 6 AM; Schedule B = treatments started at 6 PM.

†NED = no evidence of disease; DOC = died of causes other than bladder cancer; and AWD = alive with disease.

‡Patient refused further therapy and died 6 mos later (lung, bone, and liver relapses).

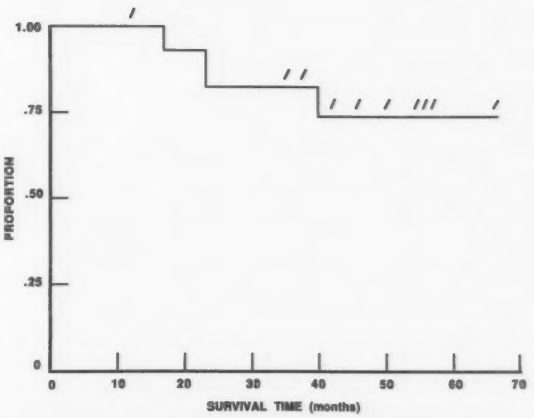

FIGURE 1.—Disease-free survival in patients receiving adjuvant chemotherapy. 10 of 13 consecutively treated patients are alive and free of bladder cancer after a median follow-up of 42 mos. Chemotherapy has been completed in each member of this group, and the median time off chemotherapy is 36 mos.

tinely performed prior to the 1st and 6th and 1 month following the 9th and/or final treatment. After completing the final chemotherapy cycle, each patient was seen at follow-up visits every 3 months for 2 years and every 6 months thereafter for the same tests listed above.

After a median follow-up period of 42 months (range, 13 to > 67), eight of the 13 patients are alive and free of bladder cancer, two are dead of other causes, and one is alive with disease. All patients have been off chemotherapy for 22–60 months.

Table 2 shows the detailed status of each patient. Eight patients received the full nine courses of treatment; only one of these patients has relapsed. Five patients did not receive all planned courses. Patient 1 refused further therapy after four uneventful cycles and died 6 months later of widespread disease. Patient 8 refused additional therapy after six courses of therapy, but her disease has not recurred. Doxorubicin administration was discontinued in Patient 9 after six treatments because of a disproportionate fall in cardiac ejection fraction. This patient died of recurrent bladder cancer 16 months after doxorubicin treatment was discontinued. Patient 12 completed only seven of the planned nine cycles of therapy because of progressive dementia. Repeated computerized tomographic examinations of the brain and cerebrospinal fluid cytologies have revealed no evidence of metastatic cancer, and this patient improved gradually and is disease-free. Treatment was discontinued for Patient 13 after four courses because of excessive toxicity; this patient has since died of other causes. Patient 11 is the only patient to die after receiving the full nine planned courses of chemotherapy. He died of a late recurrence of progressive bladder cancer 30 months after his last treatment. Figure 1 shows survival time for all patients.

Dose Intensity and Toxicity

The planned dose intensity for this monthly regimen by the method of Hryniuk and Levine (26) is 14 mg/m^2/week for each drug. The delivered dose intensity was 13.3 ± 0.3 mg/m^2/week for doxorubicin and 13.7 ± 0.3 mg/m^2/week for cisplatin. This represents 93% and 96% of planned dose intensity for these two agents, respectively. All patients experienced moderate nausea and vomiting, but it was not severe enough in any patient to stop treatment (table 3). All patients experienced a cumulative dose-related cisplatin-induced symmetrical sensory polyneuropathy, which we have described in detail elsewhere (26). It is our clinical opinion that the transient dementia which Patient 12 experienced may have been related to cisplatin administration.

Although most patients had a cisplatin-induced cumulative dose-related decrease in creatinine clearance and

TABLE 3.—Summary of toxic effects of doxorubicin and cisplatin therapy in 13 patients

Toxic effect	Adjuvant treatment group (%)
Vomiting (moderate)	13 (100)
Vomiting requiring discontinuation of treatment	0
Decrease of > 1/3 in creatinine clearance	0
Decrease in creatinine clearance requiring discontinuation of treatment	0
Granulocytopenia (< 1000 cells/mm^3)	3 (23)
Granulocytopenia associated with sepsis	1 (8)
Thrombocytopenia (< 50,000 cells/mm^3)	4 (31)
Anemia requiring rbc transfusion	5 (38)
Congestive heart failure	0

a progressive anemia, it was not necessary to discontinue or reduce the dose of cisplatin because of these toxic effects. Patient 13 was the only one for whom therapy was discontinued solely because of drug-related toxicity. This woman had severe anemia, neurotoxicity, and progressive dementia. After therapy was discontinued, a diagnosis of pernicious anemia was also made. Therapy was never reinstituted after vitamin B_{12} repletion. This patient has since died of completely unrelated causes.

Low blood cell counts were common. Total wbc counts had a mean nadir of $2700 \pm 200/mm^3$ (range, 600–8600); neutrophil counts had a mean nadir of $2530 \pm 158/mm^3$ (range, < 100–6300); and platelet counts had a mean nadir of $160 \pm 10 \times 10^3/mm^3$ (range, < 28–368). Patients treated with Schedule A had earlier recovery of all cell counts than patients receiving Schedule B, but doxorubicin dose reduction was required for < 20% of the patients. One episode of sepsis resulting from pneumonia was associated with chemotherapy-induced neutropenia. This episode required approximately 1 week of hospitalization and iv antibiotics. No patient died as a direct consequence of chemotherapy-induced toxic effects. Thirty percent of the patients demonstrated moderate thrombocytopenia. There were no major hemorrhagic complications. No patient developed symptoms of congestive heart failure during or after the doxorubicin and cisplatin treatment program. However, one patient had a substantial fall (20%) in cardiac ejection fraction and was taken off doxorubicin after six treatments. In summary, cisplatin dose reductions were not necessary and very few doxorubicin dose modifications were made. Treatments were seldom delayed. Most patients received nine monthly courses of therapy, and most recurrences were in patients who had received reduced doses or for whom therapy was prematurely discontinued.

DISCUSSION

High-dose intensity, protracted, cisplatin-based combination chemotherapy causes a complete remission of metastatic bladder cancer and a resulting prolongation of disease-free survival in up to 40% of the patients in whom it is used (18–21). Since a population at very high risk for disease recurrence and dissemination and subsequent death can be easily identified by pathological examination of the bladder, regional tissues, and lymph nodes after radical cystectomy, the use of this kind of chemotherapy in the adjuvant setting is reasonable. We chose the doxorubicin and cisplatin combination given in a high-dose intensity, circadian-timed regimen. The drug choice was based on our long-term results with this treatment in patients with metastatic TCCB (18). Fifty-six patients with unresectable locally advanced or widely metastatic TCCB were treated with this two-drug combination. Fifty-seven percent of evaluable patients responded objectively. Approximately one in four of these evaluable patients achieved complete response and three of the complete responders have been free of all cancer for at least 2 years after the cessation of all therapy. Other studies also support the effectiveness of this drug combination (19,27).

Limited data are currently available on the use of adjuvant chemotherapy to treat stage C and D_1 TCCB. In 1970, results of two studies (28,29) showed that a combination of radiation therapy and 5-fluorouracil treatment was not beneficial. In a randomized study, Daniels et al (30) treated eight patients with cyclophosphamide (600 mg/m^2), cisplatin (100 mg/m^2), and doxorubicin (60 mg/m^2). Eleven patients received cisplatin plus one to three additional drugs selected by sensitivity assay; one patient received cisplatin alone. Monthly treatment courses were given over a mean period of 3.1 months immediately after radical cystectomy. When compared to a control group of 23 patients who did not receive chemotherapy, the treated patients had a longer relapse-free interval. However, these two groups of patients did not differ in their probability of survival, which was 45% at 36 months.

Logothetis et al (31) reported superior disease-free survival following administration of adjuvant chemotherapy in 24 patients at high risk for relapse after cystectomy. These patients received five monthly courses of cyclophosphamide (650 mg/m^2), doxorubicin (50 mg/m^2), and cisplatin (100 mg/m^2). The percentage of disease-free survivors was higher with this treatment than in 46 controls: 79% versus 50% at 72 weeks. However, follow-up in this study was short. Seventy-seven percent of our high-risk patients are free of disease at a median follow-up of 3.5 years and at a median time-off-therapy of > 2.5 years. Although we have observed one very late recurrence 30 months after the ninth treatment (40 months after surgery), most patients at risk are likely to develop recurrent TCCB by the end of our follow-up period.

Each of these patients was treated with doxorubicin at one of two precisely assigned times of day; this was followed 12 hours later by cisplatin treatment. While it has been shown that circadian timing of drug administration can increase the dose intensity of doxorubicin and cisplatin therapy at a lower toxicity in patients being treated for ovarian cancer, similar conclusions cannot be drawn in this small group (25). The circadian-timed therapy may, however, have been partially responsible for the high-dose intensity achieved and the prolonged disease-free survival times (32).

To put our results in perspective it may be useful to compare them to the survival of patients in four surgical series, including a total of 179 patients who underwent radical cystectomy for stage C or stage D_1 TCCB (33–36). Five-year survival rates for patients with stage C bladder cancer range between 11% in the largest series of patients and 32% in the smallest of these four series. Five-year survival rates after resection of stage D_1 disease are between 3% and 6% (table 4). A more recent review by Herr and Yagoda (37) confirms these dismal and predictable results of radical cystectomy alone.

TABLE 4.—5-yr survival of TCCB after radical cystectomy

Investigator (yr)	Ref No.	No. of patients	Stage C	Stage D_1
Whitmore and Marshall (1962)	33	160	11%	3%
Jewett et al (1964)	34	43	12%	—
Wajsman et al (1975)	35	41	32%	0%
Richie et al (1975)	36	35	20%	6%

In summary, our data indicate that the doxorubicin and cisplatin combination at the high-dose intensity and in the rigorous circadian-timed schedules used in this study may be useful in the adjuvant setting for delaying and, perhaps, preventing local recurrence and for preventing dissemination of this highly lethal malignancy.

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Phase II Trial of a Combination of Doxorubicin and Mitoxantrone in Metastatic Breast Cancer[1]

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To exploit possible different non-cross-resistant mechanisms of cytotoxicity, 25 patients with advanced breast cancer were given combination chemotherapy consisting of iv mitoxantrone (7 mg/m^2) and doxorubicin (30 mg/m^2) every 3–4 weeks. The patients had predominantly visceral disease and received a median of six (range, one to 12) cycles of therapy. There were no complete responders, but 13 patients (52%) achieved partial remission lasting a median of 8 months (range, 4–21+). Three patients (12%) had disease stabilization and nine (36%) had disease progression. Hematologic toxicity was generally mild, with median wbc count and platelet count nadirs of 1900/mm^3 (range, 700–3100) and 160,000/mm^3 (range, 49,000–406,000), respectively. One patient may have died from treatment-related sepsis (pneumonia), but lymphangitic lung disease was not excluded. Hair loss progressing to severe alopecia over several treatment cycles was relatively common, affecting seven of 16 evaluable patients (44%). Vomiting was mild or absent in 17 (71%) of 24 evaluable patients. Three of 15 patients in whom serial measurements of left ventricular ejection fraction were performed developed significant reductions compatible with anthracycline-induced cardiotoxicity. Two of these patients also had pericardial effusions and one developed congestive heart failure. In conclusion, mitoxantrone and doxorubicin is an active, well-tolerated drug combination for the treatment of advanced breast cancer but may have appreciable cardiotoxicity. [Cancer Treat Rep 71:921–925, 1987]

Cancer of the female breast is a common malignancy occurring in almost 10% of women in some populations. Although surgery alone can cure approximately 50% of patients, the remainder develop metastatic disease which is incurable with treatments currently available. The treatment of metastatic disease has been the subject of intense investigation and considerable progress has been achieved. However, a review of the results of therapy with a wide variety of chemotherapy regimens suggests a plateau in the results obtained (1–7). Depending on patient selection, response rates to first-line drug combinations vary from 40% to 70%. However, the median response duration is usually < 1 year and reported median survival times with metastatic disease range from 18 to 24 months.

Doxorubicin is the most active single agent in the treatment of breast cancer, with a reported objective response rate of 38% in a collected series of 937 breast cancer patients with previously untreated advanced disease (8). Mitoxantrone is an anthracenedione whose structure and spectrum of activity are similar to those of doxorubicin. In a recent review of this promising new antitumor agent, an overall response rate of 33% was reported for single-agent mitoxantrone in patients with advanced breast cancer who had received no prior chemotherapy (9). Mitoxantrone has been compared with doxorubicin in several randomized crossover trials in breast cancer patients (10–12). Response rates and overall survival times did not differ significantly between the two drugs, but many studies reported a small but definite incidence of responses on crossover suggesting a degree of non-cross-resistance. In addition, there is some evidence from in vitro studies that the cytostatic mechanisms of mitoxantrone and doxorubicin are different (13,14). With regard to side effects, myelosuppression is dose-limiting but the incidence of nausea, vomiting, alopecia, stomatitis, and possibly cardiotoxicity is significantly reduced with mitoxantrone (9,11).

The above considerations prompted us to study the effectiveness of combined mitoxantrone and doxorubicin in patients with advanced breast cancer. Because myelosuppression occurs with both compounds, only moderate doses were given in this phase II trial. In addition, the possibility of the combination causing synergistic car-

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diac damage was taken into account in the design of the study.

PATIENTS AND METHODS

A total of 25 patients with histologically proven advanced breast cancer were entered in this study. Twenty patients had strictly measurable visceral or soft tissue disease and five patients had evaluable bone lesions as the sole manifestation of metastasic disease. Previous cytotoxic therapy as an adjuvant or as treatment for metastatic disease did not exclude patients from entry provided that the therapy had not included anthracyclines. A history of myocardial infarction within the previous 6 months, heart failure requiring regular medication, severe angina, or a left ventricular ejection fraction $< 50\%$ excluded patients from the trial. Patients with receptor-positive or -unknown tumors had to have received an adequate trial of hormonal treatment.

Therapy comprised iv mitoxantrone (7 mg/m^2) and doxorubicin (30 mg/m^2) on Day 1, repeated every 3-4 weeks. The drugs were administered sequentially by rapid push injection into the side-arm of a rapidly running iv infusion. The dose selected for each drug of the combination represented approximately 50% of the standard single-agent dosage. An initial dose reduction of 20% was employed in eight patients for reasons which included bone marrow involvement with peripheral blood cytopenias, age > 70 years, low performance status, and extensive prior radiotherapy. As an antiemetic, patients received dexamethasone (10 mg iv) prior to treatment. Patients generally continued on therapy until disease progression but, because of the possibility of additive cardiotoxicity with the combination, an arbitrary maximum ceiling of 12 treatment cycles was imposed.

Baseline studies included history and physical examination, Eastern Cooperative Oncology Group performance status, tumor measurements, cbc, biochemical profile, serum carcinoembryonic antigen (CEA) level, chest x-rays (CXR), bone scan, and, where appropriate, skeletal survey, computerized tomographic scan(s), and liver scan. Nadir blood cell counts were obtained on Days 10 and 14 of the initial two to three treatment cycles. Cbcs, biochemical profiles, and CEA levels were obtained prior to every cycle. CXRs and bone scans were repeated every three cycles together with all other scans which were being used in disease evaluation. Additional unscheduled investigations were performed as necessary to document suspected disease recurrence or progression.

Cardiac function was monitored by serial estimations of the left ventricular ejection fraction using multigated analysis (MUGA). The lower limit of normal for this investigation at our institution is 50%. MUGA scans were generally performed prior to entry in the study and every three treatment cycles thereafter. Tumor response and drug toxic effects were graded according to World Health Organization (WHO) criteria (15). The duration of partial response was measured from the first day of treatment to the date of first observation of progressive disease. Survival was measured from the first day of treatment with the combination.

RESULTS

Clinical characteristics of the 25 patients are shown in table 1. Although only 11 patients had either received prior chemotherapy for metastatic disease or had relapsed during or within 1 year of completing an adjuvant chemotherapy program, this was a prognostically relatively poor-risk group of patients in that 18 patients had visceral-dominant disease. A total of 15 patients had received chemotherapy at some stage: eight as adjuvant therapy (melphalan and 5-fluorouracil), five as treatment for advanced disease (CMF; cyclophosphamide, methotrexate, and 5-fluorouracil), and two as therapy for both adjuvant and advanced disease. The median number of cycles of CMF was nine (range, four to 17).

The 25 patients were given a median of six courses of treatment (range, one to 12). Thirteen patients (52%) achieved partial response and three (12%) had disease stabilization (table 2). Nine patients (36%) showed disease progression. Considering only the 14 relatively good-risk patients, defined as patients who had received either no prior therapy, hormonal treatment alone, or remote adjuvant chemotherapy, nine (64.3%) achieved partial remission, three (21.4%) had disease stabilization, and only two (14.3%) showed disease progression.

There were no complete responders to chemotherapy but one patient with locally advanced disease was converted to a complete response with radiotherapy after chemotherapy had induced marked regression of her breast mass. Three patients with bone disease as the

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	25
Median age in yrs (range): 55 (30-76)	
Performance status	
0	1
1	11
2	8
3	5
Dominant site of disease	
Soft tissue	2
Bone	5
Visceral	18
Prior therapy	
Hormones	7
Adjuvant chemotherapy alone	8*
Chemotherapy for advanced disease	7†
None	3

*4 patients relapsed during or within 1 yr of completing adjuvant chemotherapy.

†2 patients had also received (remote) adjuvant chemotherapy.

TABLE 2.—Treatment results*

Results	All patients	Good-risk group†
Partial response	13 (52)	9 (64.3)
Stable disease	3 (12)	3 (21.4)
Progressive disease	9 (36)	2 (14.3)
Total	25 (100)	14 (100)
Median duration of partial response in mos (range)	8 (4-21+)	6 (4-21+)
Median survival in mos (range)	12 (1-30+)	12 (1-30+)

*Unless otherwise specified, values = No. of patients (%).
†Excluding 7 patients with prior chemotherapy and 4 patients who relapsed within 1 yr of adjuvant chemotherapy.

sole manifestation of metastatic cancer responded. The low patient numbers preclude a more detailed analysis but responses were seen at all other sites of metastatic involvement. A median of three treatment cycles (range, two to seven) were administered before partial response was documented. Considering all 25 patients, the median duration of partial response was 8 months (range, 4-21+). Median survival was 12 months (range, 1-30+) (table 2). Four patients are still alive 21, 22, 29, and 30 months after starting the combination. In table 3 response rate, duration of partial remission, and survival are listed according to whether the patients had received prior chemotherapy, prior treatment for advanced disease, or prior adjuvant chemotherapy.

Hematologic toxicity encountered with this drug combination was generally modest. Median wbc count nadir was 1900/mm^3 (range, 700–3100) and median platelet count nadir was 160,000/mm^3 (range, 49,000–406,000). A 75-year-old woman with widespread metastatic disease died with possible pneumonia acquired while leukopenic (wbc count nadir, 700/mm^3), although the CXR appearances were equally compatible with lymphagitic lung metastases. An additional patient required hospital admission for treatment of leukopenia-related sepsis,

TABLE 3.—Treatment results according to prior therapy*

Results	Prior chemotherapy†	Prior treatment for advanced disease‡	Prior adjuvant therapy§
Partial response	7	9	6
Stable disease	1	3	1
Progressive disease	7	5	3
Total	15	17	10
Median duration of partial response in mos (range)	6 (5-8)	8 (4-21+)	7 (5-8)
Median survival in mos (range)	12 (4-29+)	12 (4-30+)	12 (4.5-16)

*Unless otherwise specified, values = No. of patients.
†Adjuvant, 8 patients; advanced disease, 5 patients; and both, 2 patients.
‡Hormones, 10 patients; and chemotherapy, 7 patients.
§All patients received melphalan and 5-fluorouracil.

TABLE 4.—Nonhematologic toxic effects

	No. of patients (%) with —	
	Hair loss	Nausea and vomiting
WHO grade		
0	2 (8)	13 (52)
1	3 (12)	4 (16)
2	4 (16)	6 (24)
3	7 (28)	1 (4)
Not evaluable	9 (36)	1 (4)
Total	25 (100)	25 (100)

with wbc count of 1000/mm^3. Dose reductions were required in only three of the 17 patients beginning therapy at full doses. Two of these patients had received prior chemotherapy: one received adjuvant melphalan and 5-fluorouracil 5 years previously and one had received ten cycles of CMF. Conversely, dose escalations were possible in two of the eight patients who began therapy at a reduced dose because of medical reasons. Treatment delays to allow recovery of peripheral blood cell counts were infrequent.

Details of other noncardiac toxic effects are shown in table 4. Alopecia was already present in a significant number of patients due to prior chemotherapy or radiotherapy and was therefore not evaluable in these patients. Hair loss tended to be progressive with each course and was maximal in some patients only after four or five treatment cycles. Therefore, no attempt was made to grade alopecia in patients receiving fewer than four courses of treatment. Nausea and vomiting were minimal, with only a single patient reporting severe symptoms, possibly on a psychogenic basis.

Serial estimations of the left ventricular ejection fraction by MUGA scan are available in 15 patients. The median pretreatment ejection fraction measured 69% (range, 46%–86%) and the median posttreatment value was 66% (range, 34%–75%). The posttreatment value represents the average recorded after a median of six (range, two to 12) treatment cycles. Three patients developed significant reductions in left ventricular ejection fraction compatible with anthracycline-induced cardiotoxicity. One patient, a 60-year-old woman, was entered in the trial even though her ejection fraction measured only 46% several months earlier. This low value may have reflected underlying ischemic heart disease though she also had a pericardial effusion. After two treatment cycles, at a time when she had progressive disease, a repeat ejection fraction measured only 34%. She developed mild congestive heart failure and died shortly thereafter with progressive disease. It is possible that this patient's deteriorating cardiac status was due to an increase in the size of her pericardial effusion, particularly since she had progressive disease elsewhere. The second patient, a 39-year-old woman, developed a fall in ejection fraction from 68% to 46% after receiving the maximum planned number of 12 treatment cycles.

Twenty-one months after beginning therapy she continues in partial remission with an abnormal (though stable) bone scan and has no cardiac symptoms. The third patient, 50 years of age, achieved partial remission of disease which included a pericardial effusion but was taken off study after seven treatment courses when her ejection fraction fell to 44% from a pretreatment value of 58%. She did not develop other signs or symptoms of cardiac dysfunction.

DISCUSSION

The rationale for conducting this trial of combination chemotherapy was that evidence exists both experimentally and clinically that doxorubicin and mitoxantrone are at least partially non-cross-resistant (10–14). The results demonstrate, albeit in a relatively small number of patients, that the combination is probably as effective as any other currently employed in the treatment of metastatic breast cancer.

During this trial, hematologic toxicity was relatively mild and a modest increase, perhaps of the order of 20%–30%, in the dose of both agents would probably be feasible. Alternatively, a third drug could be added, perhaps even another doxorubicin analog. It would also be of interest to determine the effect of giving the drugs separately at a higher dosage on Days 1 and 8 of a 4-week treatment cycle. The majority of patients in this trial had visceral-dominant disease and additional studies should be drawn from a more representative patient population.

Other noncardiac toxic effects associated with this drug combination were also relatively mild. Nausea and vomiting of the degree commonly associated with standard-dose doxorubicin therapy were reported by only a single patient. Alopecia severe enough to require a wig was more frequent, occurring in approximately half the patients. Overall, the degree of hair loss was probably intermediate between what can be expected following treatment with standard single-agent dosages of either mitoxantrone or doxorubicin.

Cardiac toxicity was more problematic. Three of the 15 patients in whom serial MUGA scans were performed showed a significant fall in left ventricular ejection fraction while receiving treatment. The diagnosis of anthracycline cardiotoxicity seems highly likely in the 39-year-old patient who received 12 treatment cycles with the combination. The two other patients both had pericardial effusions. Since pericardial effusions can reduce left ventricular ejection fraction, the relative contributions of malignant pericardial disease and drug-induced toxicity to the fall in ejection fraction are uncertain. The patient numbers in this trial are obviously too small to reliably estimate the true frequency of cardiotoxicity. However, in view of our findings it would seem prudent to monitor cardiac function carefully in any future studies of combination anthracycline treatment.

Previous studies in metastatic breast cancer have shown that overall treatment results with relatively low-dose chemotherapy programs compare favorably with more aggressive regimens (16,17). Although a positive correlation between treatment outcome and the intensity of therapy administered has recently been reported, metastatic breast cancer is ultimately fatal in all patients regardless of the dose and treatment selected (18). Significant improvements in the management of this disease probably must await the discovery of completely new drugs or the introduction of radically different treatment approaches. Until that time the routine management of metastatic breast cancer (ie, outside the setting of randomized clinical trials) should be palliative in intent and should employ active regimens which produce only modest toxicity. Provided that the apparent high incidence of cardiotoxicity is not confirmed, the combination described here is well-suited for this purpose.

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Effects of the Mode of Administration of Mitomycin on Tumor and Marrow Response and on the Therapeutic Ratio[1,2]

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The antineoplastic and toxic effects of mitomycin (MC) administered as a single pulse injection were compared with the effects of MC given as infusions over 2, 5, or 24 hours and with the effects of the drug administered in fractionated regimens incorporating 2–20 injections. The antineoplastic effects of MC were assessed using EMT6 mouse mammary tumors implanted in BALB/c mice; tumor response was assessed using both cell survival and tumor growth assays. The host toxicity of MC was assessed by measuring the survival of bone marrow stem cells (CFU-S) and by determining $LD_{50/30}$'s (which reflect primarily marrow injury). The survival curve for cells from tumors treated with varying doses of MC was exponential, with no shoulder which might suggest accumulation of sublethal lesions. No evidence was found that the tumor cells repaired MC damage when left in situ for up to 24 hours after treatment (repair of potentially lethal damage) or that the tumor repaired sublethal damage during times of up to 24 hours between drug treatments or during drug infusions. The responses of tumors treated with single injections of MC, with infusions over 2, 5, and 24 hours, with two injections separated by 5 or 24 hours, and with five daily injections were all similar. The survival curve for CFU-S from mice treated with varying doses of MC was exponential, with no shoulder which might suggest accumulation of sublethal lesions. $LD_{50/30}$'s were the same whether MC was administered as single injections, as infusions over 2, 5 or 24 hours, or as five daily fractions. Longer fractionated regimens, in which mice received daily injections, 5 days per week, for up to 4 weeks, produced less host toxicity but also less antineoplastic effect; the therapeutic ratio for the most protracted regimen tested was lower than that for single injections. The therapeutic ratios achieved with infusions and fractionated regimens were never greater than that obtained with a large single dose, confirming clinical impressions that infrequent, large doses of MC are an optimal method of treatment with this drug. [Cancer Treat Rep 71:927–934, 1987]

The antineoplastic antibiotic mitomycin (MC) has been used for > 20 years as a component of multi-agent cancer chemotherapy regimens (1–3). More recently, we and others have been examining the potential of MC as an adjunct to radiotherapy (4–18), on the theory that the selective toxicity of radiation to aerobic cells and the preferential toxicity of MC to hypoxic cells should combine to make regimens which include both agents unusually effective for the treatment of solid tumors (5,6,10). Preliminary results of the first clinical trial of this combination at Yale (15), designed on the basis of our laboratory studies, are extremely promising. However, more data are needed to optimize the combination of radiotherapy and MC, as laboratory studies examining combinations of x-rays and MC generally have shown only additive or slightly supraadditive antineoplastic effects (9,13,18), possibly as a result of inhomogeneous distribution of active drug within the tumor. Attempts to improve combination regimens have examined a number of possible approaches, including modulating the metabolism of MC to improve its selectivity and developing related bioreductive alkylating agents with greater selectivity (14,16,17). The experiments reported here were performed to determine whether protracted or fractionated regimens of treatment with MC might yield a greater therapeutic ratio than the large pulse injections tested in our earlier laboratory studies and in our ongoing clinical trial.

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MATERIALS AND METHODS

Animals

BALB/c KaRw mice 2.5–3 months of age were used throughout these experiments. These specific pathogen-free animals were bred and maintained in a barrier in our institution as described elsewhere (9,19) and were monitored routinely to define their microbiological status.

Tumor Cells

EMT6 cells (subline EMT6-Rw) were maintained by alternate passage in mice and in cell culture using the techniques described previously (9,19,20); the cells and tumors were routinely tested and found to be free of contamination by mycoplasma, bacteria, or pathogenic viruses. Experimental tumors were implanted by injecting 2×10^5 cells, harvested from exponentially growing cell cultures, intradermally in the flank as described previously (19). Tumors were used for experiments when they reached 100 mm^3 in volume, approximately 2 weeks after implantation.

Tumor Growth Curves

Tumor growth was followed by measuring the three diameters of the neoplasms three to five times per week with vernier calipers. Tumor volumes were calculated from the three diameters, assuming that the tumors were hemiellipsoidal (19,20). The growth curve for each tumor was examined and used to determine the time necessary for the tumor to grow from the treatment volume ($\sim$ 100 mm^3) to four times the treatment volume ($\sim$ 400 mm^3). The mean time for each group was calculated from the values for the individual tumors. This method of analysis has been shown to avoid certain pitfalls inherent in analyses based on average tumor growth curves (21,22). Tumor growth delays produced by different treatment regimens were compared using Student's t test.

Analysis of Tumor Cell Survival

The viability of cells suspended from treated tumors was assayed by determining their ability to form colonies in cell culture as described previously (19,20,23). Mice were killed 2 hours after the final injection of MC or 1 hour after the end of the MC infusion, and their tumors were removed, minced, and trypsinized to form a single-cell suspension. The suspended cells were counted, using trypan blue to identify cells killed during the suspension process, plated in tissue culture dishes in Waymouth's medium supplemented with 15% fetal bovine serum, and incubated for 2 weeks to allow the viable (clonogenic) cells to form macroscopic colonies. The survival of cells in treated tumors was calculated by comparing the clonogenicity of cells from treated tumors with that of cells from untreated tumors assayed on the same day; plating efficiencies for cells from control tumors were generally between 30% and 40%. The numbers of cells suspended from treated and control tumors were also examined to ensure that there was no rapid loss of dead cells, which might compromise the cell survival measurements (9,23–25); no changes in cell yield were observed with the intervals between drug treatment and assay of 2–24 hours used in these studies. Cell survivals after different treatment regimens were compared using Student's t test.

Analysis of the Survival of Marrow Stem Cells

To measure the viability of the hematopoietic progenitor cells, the right femurs of the mice were removed and the marrow was flushed from the femur with a 25-gauge needle and 2 ml of McCoy's 5A medium. The trypan-blue-excluding nucleated cells in the resulting single-cell suspension were counted with a phase contrast microscope. The viability of the uncommitted progenitor cells was assayed using the spleen colony technique first described by Till and McCulloch (26). Recipient mice were irradiated whole body with a dose of 7 Gy 24 hours before iv injection of the marrow cells. Mice were treated and maintained in the barrier to guard against infection. Recipients were killed 10 days after injection and the spleens were removed, fixed in Bouin's solution, and washed with ethanol. Colonies on the spleens were counted using a dissecting microscope (20×). Recipients of control suspensions were injected with 2×10^4 cells; this yielded 7.2 ± 1.1 colonies per spleen in the experiments reported here. Recipients of cells suspended from treated mice were injected with larger numbers of cells, expected to produce similar numbers of spleen colonies; where cell survival could not be predicted accurately, different dilutions were injected into different groups of eight to ten recipients, and the dilution yielding ten to 30 colonies per spleen was used in the analysis.

Analysis of Host Toxicity

The toxicity of MC given in protracted regimens was assessed by determining the $LD_{50/30}$, which is thought to reflect primarily hematologic toxicity. Deaths occurred at $\sim$ 10 days with single MC treatments at doses near the LD_{50}. At supralethal doses, deaths occurred earlier ($\sim$ 7 days at 10–13 $\mu g/g$), probably reflecting the contribution of intestinal damage to the toxicity (27). The timing of the deaths was similar to that reported for BALB/c mice dying from irradiation lethality (28). The LD_{50}'s were used in these studies in lieu of the analyses [bone marrow stem cell (CFU-S) survival, colony-forming unit-granulomonocytic (CFU-GM) survival, or the sequential monitoring of peripheral blood cells] generally used in our laboratory to monitor the hematologic effects of irradiation or bioreductive alkylating agents, because cell proliferation, cell death, and cell loss during the protracted fractionation regimens used in these studies would make interpretation of data obtained with these other endpoints exceedingly problematic. Animals in these studies were maintained so as to guard against

intercurrent infections, and were generally examined two to three times per day. Animals were followed for 30 days after the end of the MC treatments. Animals were euthanized humanely when they showed physical or behavioral evidence of terminal toxicity. Toxicity data were analyzed using computerized probit analysis techniques. Comparisons of toxic effects produced by different therapeutic regimens were based on the best fit probit lines and the error limits for lines and the LD_{50}'s (29).

Drug

MC was provided by Bristol-Myers Company, Syracuse, NY. The drug was dissolved in warm physiologic saline, was protected from light to avoid photodegradation, and was used within 1 week of preparation. In most studies, MC was administered by rapid ip injection. In a few studies, as noted in the text, MC was administered by continuous infusion over 2, 5, or 24 hours. In these studies, heparinized catheters filled with saline or with MC in an appropriate concentration were implanted ip. Mice were allowed to recover from the anesthesia and the appropriate saline or drug solution was infused continually through the catheter at the appropriate rate using a Sage-Adams syringe pump. This technique is a variation of that used in our laboratory for many years in studies requiring continuous infusions through iv catheters; except for the position of the catheter in the mouse, the technique is similar to that described by Brown and Goffinet (30). The technique used for these infusions allows the animals to move freely around their enclosures and to have free access to food and water throughout the treatment.

RESULTS

MC is effective in killing the cells of solid EMT6 tumors (fig 1). The dose-response curve relating the survival of cells suspended from solid tumors to the dose of MC given as a single ip injection could be well fitted by an exponential function (ie, the log survival vs dose relationship in fig 1 is well fit by a straight line). Comparisons of survival curves for cells from tumors explanted 2 and 24 hours after injection provided no evidence for repair of potentially lethal damage after MC treatment. Repair of potentially lethal damage after irradiation or drug treatment increases the survival measured several hours after treatment over that seen when cells are suspended soon after treatment, decreasing the slope of the measured dose-response curve (24,25). In contrast, survival curves measured 2 and 24 hours after treatment of EMT6 tumors with MC were indistinguishable (fig 1).

The effect on the tumor cell survival of protracting the MC treatment was tested by infusing the drug over 2, 5, or 24 hours. The effect of fractionation was assessed by dividing the MC dose into two injections separated by 5 or 24 hours. As shown in figure 2, neither infusing the MC over a longer period of time nor dividing the

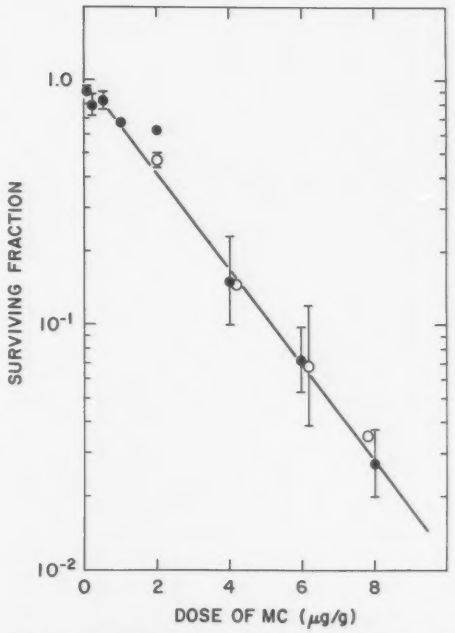

FIGURE 1.—Survival of cells suspended from EMT6 tumors after treatment with a single injection of MC. Closed circles = tumors suspended 2 hrs after injection; open circles = tumors suspended 24 hrs after injection; points with error limits = means ± SEM of survivals determined in 3–20 experiments; points without error limits = means of 2 determinations; and line = survival curve, determined by regression analysis of 2-hr data.

dose evenly between two injections altered the survival of the tumor cells significantly from that seen with a single injection. These findings suggest that, for EMT6 tumor cells in vivo, repair of sublethal damage during prolonged MC infusions or between pulse MC injections is not a factor influencing the response of the tumor cells to protracted or fractionated treatments up to 24 hours in duration.

The effects of fractionated MC treatments on tumor growth were also examined (fig 3, table 1). This endpoint was used for two reasons. First, use of a tumor cell survival endpoint to examine the response of tumors to very protracted treatment regimens is extremely problematic, as cell proliferation and loss of drug-killed cells during treatment will compromise measurements of cell survival performed with cells suspended from the tumors after treatment. Tumor growth and cure endpoints are therefore more appropriate for use in examining very protracted treatment regimens. Second, the assumptions implicit in the use of in vitro cell survival assays and the potential artifacts associated with these cell survival assays are very different from the assumptions and potential artifacts inherent in the use of tumor growth assays (22); use of both assays to examine the same biological questions therefore ensures that

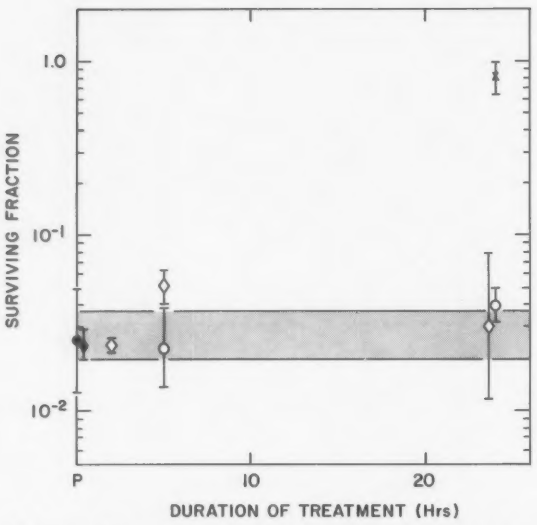

FIGURE 2.—Effect on tumor cell survival of protracting or fractionating treatment with a total dose of 8 μg/g of MC. Open diamonds = MC infused over 2, 5, or 24 hrs; closed diamond = single ip injections from the same set of experiments; open circles = 2 ip injections of 4 μg/g/injection separated by 5 or 24 hrs; closed circle = corresponding single-dose controls; and X = sham-control mice, infused with saline for 24 hrs. Points are means ± SEM for ≥ 4 independent determinations. Stippled area = SEM of pooled single-injection treatment data from both series of studies. None of the survivals for fractionated or protracted treatments differ significantly from the survivals after pulse treatments.

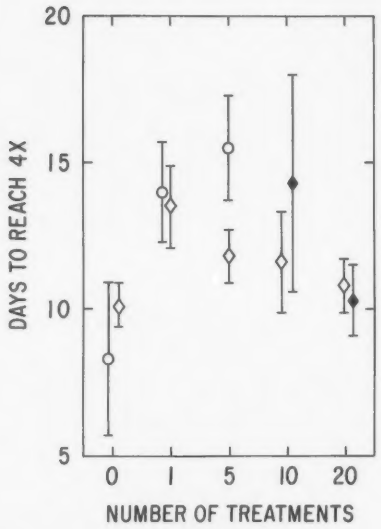

FIGURE 3.—Effects of fractionated MC treatments assessed by a tumor growth endpoint. The time for tumors to grow from the volumes at the time of treatment (~ 100 mm^3) to 4 times the treated volumes is shown for each treatment condition. Open circles and diamonds = mice that received 6 μg/g of MC in 1, 5, 10, or 20 ip injections. Closed diamonds = mice that received 10 or 20 treatments at total dose equitoxic to a single dose of 6 μg/g. Circles and diamonds represent different experiments. Points are means ± SEM's.

the phenomena observed are real and independent of the measurement technique.

A large dose of MC (8 μg/g) was used to examine the effects on tumor growth of two fractions of MC. The results of this experiment were complicated by the fact that this dose of MC resulted in some deaths among the treated mice; toxic effects were similar in all treatment groups (table 1). Mice dying of drug toxicity were deleted from the data set used to examine tumor growth delay. The results of this study confirm findings obtained using a cell survival endpoint: a single treatment with 8 μg/g and regimens including two doses of 4 μg/g separated by 5 or 24 hours all produced similar retardation of tumor growth (table 1). Therefore these tumor growth data, like the cell survival data, provide no evidence that EMT6 tumors repair sublethal damage between MC treatments separated by up to 24 hours.

To examine further the effects of fractionation on tumor response, the growth of control tumors was compared with the growth of tumors receiving a total dose of 6 μg/g MC as a single injection or divided into 5, 10, or 20 equal fractions, administered once per day/5 days per week (fig 3). The single dose of 6 μg/g MC, chosen to avoid the toxicity encountered with the 8 μg/g dose described above, produced only a moderate delay in the growth of the tumors. The efficacy of the drug was not significantly different when this dose was divided into five or 10 fractions, but was less when the same dose was divided into 20 fractions. Groups of mice were also treated with MC in 10- and 20-fraction regimens with doses of MC that were expected to be equitoxic to 6 μg/g on the basis of the toxicity studies shown in fig 4; these mice received 10 treatments with 0.93 μg/g/fraction or 20 treatments of 0.67 μg/g/fraction (ie, ¾ of the LD_{50}'s determined from the experiments shown on fig 4). The effects of the 10-fraction regimen were not significantly different from that of the equitoxic single dose. The 20-fraction regimen produced no significant alteration of tumor growth from that seen without treatment. In these studies, protraction of MC treatment using frac-

TABLE 1.—Comparison of tumor growth after treatment of mice with 8 μg/g of MC given as a single dose or divided into 2 doses given with a separation of 5 or 24 hrs*

Treatment	Days to reach 4 × (Mean ± SEM)	Deaths from toxicity No. of animals
None	11.2 ± 1.1	0/10
8 μg/g	21.4 ± 0.9	2/9
2 × 4 μg/g, 5 hrs	22.6 ± 5.5	2/9
2 × 4 μg/g, 24 hrs	20.9 ± 1.6	2/9

*The growth delays induced by the 3 MC regimens are not significantly different.

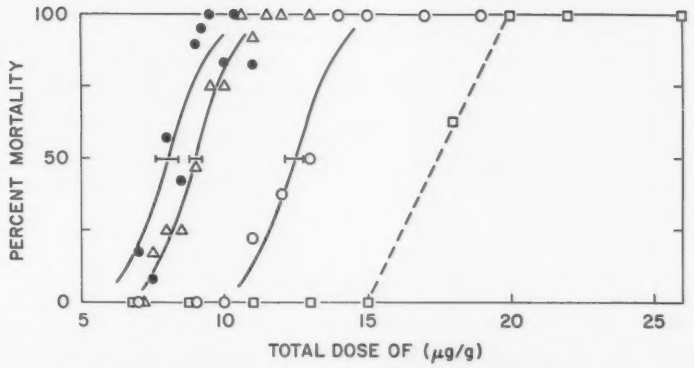

FIGURE 4.—Toxicity of fractionated MC treatments to BALB/c mice. Mice were given 1 (●), 5 (△), 10 (○), or 20 (□) doses. Animals receiving multiple doses received 1 treatment/day, 5 days/week. Solid lines were determined by computerized probit analyses. Bars are LD_{50}'s with standard deviations, determined by probit analysis (27). Dashed line = estimated dose-response curve; data not amenable to probit analysis.

tionated regimens did not produce therapeutic gain. In fact, the therapeutic ratio for the highly protracted, 20-fraction/26-day regimen was lower than that for large single doses (ie, *less* antineoplastic effect was obtained for an equal level of toxicity).

The effects of MC on the hematopoietic system were also examined. The dose-response curve relating the survival of the spleen colony forming progenitor cells (CFU-S) to the dose of MC could be well fitted by an exponential function (fig 5), suggesting that the CFU-S did not accumulate sublethal damage as a result of MC treatment and that repair of sublethal damage should not be expected to be a factor influencing the response of the marrow during protracted or fractionated regimens.

The toxic effects of MC given as a single ip injection, in 5 equal daily fractions, in 10 equal fractions (5 days/week for 2 weeks), and in 20 equal fractions (5 days/week for 4 weeks) were compared (fig 4). The 10- and 20-fraction regimens were much less toxic than the single treatments, although an exact LD_{50} could not be calculated for the 20-fraction data using probit analysis techniques because mixed responses were observed at only one dose (29). The LD_{50}'s for the single dose and the 5-fraction regimen differed by $< 10\%$. These data suggest that there is little hematologic recovery from sublethal MC damage by the critical stem cells between the daily fractions of the 5-fraction regimen. The longer overall treatment times in the 10-fraction/12-day and 20-fraction/26-day regimens probably allowed proliferation to occur during therapy, increasing the number of hematologic stem cells and therefore increasing the resistance of the animals. Proliferative or slow repair processes during these long regimens could also lead to lower lesion densities in the drug-injured cells during the later treatments, and therefore to increased resistance of the stem cells during these regimens.

The toxic effects of MC given as a single injection and as a continuous infusion over 2, 5, or 24 hours were all similar (fig 6), as evidenced by the overlap in the data sets, by the similarity of the calculated probit curves, and by the error limits of the LD_{50}'s. This suggests that there is little or no repair of sublethal MC damage by the critical hematopoietic stem cells during infusions of up to 24 hours in duration.

In summary, the data in figs 4 and 6 show that neither protracting a MC treatment over 2–24 hours by infusion nor dividing the treatment into multiple injections over 5 days produced major changes in drug toxicity. Longer treatment courses, which would allow proliferation of critical stem cell populations during treatment, were necessary to decrease the toxicity of the drug.

DISCUSSION

The experiments described in this report examined the response to MC of a transplanted mouse tumor and a dose-limiting normal tissue, the marrow. One purpose of these studies was to assess whether either protraction of the MC treatment over several hours by the use of infusions or fractionation of the MC treatment among several injections would improve the therapeutic ratio and allow a greater antineoplastic effect to be achieved at a similar level of normal tissue toxicity. The data provide no evidence that protraction or fractionation of MC treatment produced therapeutic gain. Moderately protracted regimens produced toxic effects and antineoplastic effects similar to those of single treatments with the same total dose. Longer fractionation regimens produced less host toxicity, but also less antineoplastic activity. For the longest fractionation regimen tested (20 fractions given over 4 weeks) the therapeutic ratio was less than that obtained with a large single dose;

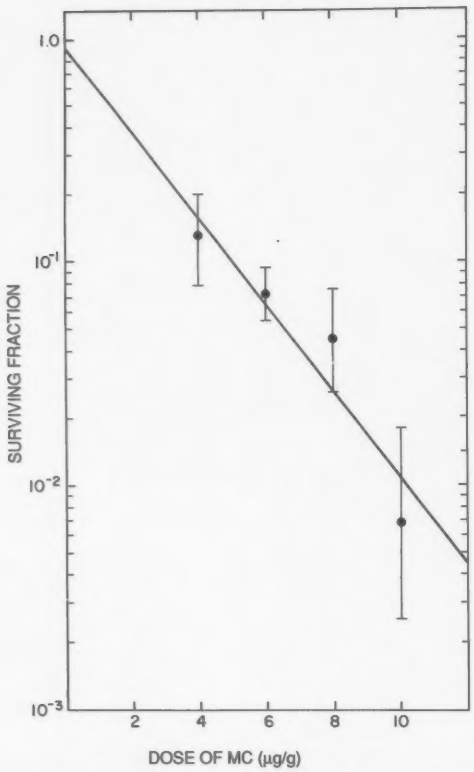

FIGURE 5.—Survival of bone marrow stem cells (CFU-S) from mice treated with different doses of MC. Points = means ± SEMs from ≥ 3 independent determinations. Line = survival curve, determined by regression analysis.

that is, a dose giving an equal level of host toxicity produced less antineoplastic activity.

In these studies the response of the tumors to MC would be dominated by the response of the aerobic tumor cell population, because 80% of the viable tumor cells are aerobic, while only 20% are hypoxic (20). In vitro data suggest that hypoxic cells metabolize MC differently than aerobic cells, are more sensitive to killing by MC than aerobic cells, and may be damaged by different lesions than those killing aerobic cells (4-7,11-14). It is therefore possible that the response of the hypoxic tumor cell population would be different from the response of the overall tumor cell population defined in these experiments. Additional experiments, using a different experimental design, will be necessary to examine the response of hypoxic cells; these are in progress.

Another purpose of these studies was to assess the effects of repair of sublethal and potentially lethal damage in determining the response of tumors and the hematologic system to MC. In EMT6 tumors, cell survival assays could be used to test for accumulation and repair of sublethal damage and for repair of potentially lethal damage. The exponential shape of the dose-response curve provided no evidence that tumor cells accumulated sublethal damage from an injection of MC. Rapid repair of sublethal damage would have resulted in an increase in the tumor cell survival when a MC dose was divided into two fractions separated by 5 or 24 hours (to allow repair to occur between fractions) or was administered as an infusion over 2, 5, or 24 hours (to allow repair to occur during treatment). Neither of these manipulations altered the survival of the tumor cells, suggesting that the tumor cells did not repair sublethal lesions resulting from MC treatment within this time period. This conclusion was verified using a tumor growth endpoint; the growth perturbations produced by MC were similar, regardless of whether the drug was delivered as a single injection or divided into two equal doses separated by 5 or 24 hours. If repair of sublethal MC damage occurs in EMT6 tumor cells, the time course of this repair must be much slower than that observed for treatment of these cells with irradiation (31,32).

Cell survival studies were also performed to test for repair of potentially lethal damage after treatment of tumors with MC. The survival curve measured for EMT6 cells explanted soon after treatment was identical to that for cells explanted 24 hours after treatment. This is in agreement with previous observations (9), which were limited to comparing the effects of a single dose (6 μg/g) at different times after treatment. These data provide no evidence that EMT6 tumors in vivo repair potentially lethal MC damage during the first 24 hours after treatment.

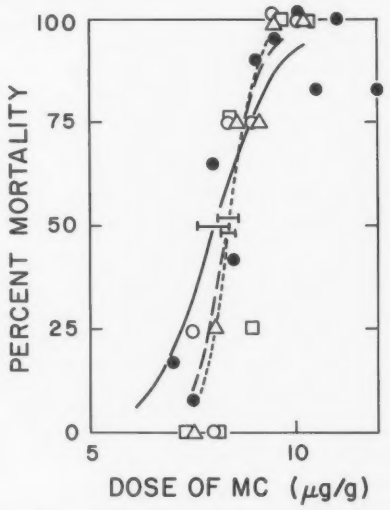

FIGURE 6.—Toxicity of protracted MC treatments to BALB/c mice. MC was given either as a single ip injection (●) or as an infusion over 2 (○), 5 (△), or 24 (□) hrs. Curves were determined by probit analysis. The control data are replotted from fig 4. The 24-hr data were not amenable to probit analysis. Bars are LD_{50}'s with standard deviations, determined by probit analysis (27).

These in vivo findings are in agreement with our cell culture data, which provided no evidence for the repair of sublethal or potentially lethal damage by exponentially-growing or plateau phase EMT6 cells after treatment with MC in vitro (8,9,12). Our findings are in contrast with reports of repair of MC damage by other cell lines in vitro (33,34). The time course of repair in these cell lines was rapid, and essentially complete in < 24 hours; such repair should have been detected in our experiments, if it occurred in the cells examined. EMT6 cells in vivo and in vitro are extremely proficient in the repair of sublethal and potentially lethal damage after treatment with ionizing irradiation and with certain other chemotherapeutic agents, including bleomycin, cyclophosphamide, and 5-fluorouracil (24,25,31,32). Repair of sublethal and potentially lethal damage after treatment of EMT6 cells and tumors with irradiation and drugs is generally rapid, and is often complete within 3–6 hours after treatment. The absence of rapid repair of sublethal or potentially lethal damage repair after MC suggests that the lesions producing cytotoxicity after treatment of EMT6 cells with MC are qualitatively different than the lesions produced by irradiation and these other drugs.

Repair is more difficult to assess in the hematopoietic system, as the rapid cytolysis which occurs after treatment of hematologic cells with many agents compromises the use of cell survival assays to assess the effects of delayed plating and fractionated treatments. Analyses of cell yield cannot be used to circumvent this problem, as the stem cells constitute only a very small fraction of the nucleated cells suspended from the marrow and cannot be identified and enumerated separately; there is therefore no way to analyze the changes in the number of total (viable plus sterilized) stem cells after treatment. Because of this, our observations on the repairability of MC damage in the marrow are limited. The survival curve for the CFU-S explanted shortly after MC treatment is exponential (fig 5), providing no evidence of a shoulder which might suggest an accumulation of sublethal drug damage. An exponential survival curve was also found by Lahiri (35) for CFU-S in CPB/s mice treated with MC. The $LD_{50/30}$, which is thought to reflect primarily death due to hematologic damage, is the same whether MC is given as a large single dose or as infusions over 2, 5, or 24 hours, suggesting that repair of drug damage during these infusions does not increase the resistance of the hematologic stem cells. The fact that the $LD_{50/30}$ is only slightly increased when MC is divided into five daily treatments, rather than given in a single pulse treatment, also suggests that little or no repair of sublethal damage occurs during this period.

With longer fractionation regimens (10 fractions over 12 days and 20 fractions over 26 days), both the toxicity and the antineoplastic effect of MC are diminished. This probably reflects an increase in the number of clonogenic tumor and marrow stem cells, caused by cell proliferation occurring during these very long treatment regimens. In this system, however, it would appear that proliferation produced a greater effect in the tumor than in the marrow, and resulted in a decrease in the therapeutic ratio during highly protracted regimens.

Data obtained with this animal model system therefore support clinical impressions that large infrequent doses of MC may be more efficacious than highly fractionated treatments (3) and are in agreement with other data showing that the hematologic toxicity of MC is not decreased by protracting treatments over relatively short periods using multiple injections or slow release systems (36). The nonrepairable nature of the lethal MC lesion may be in part responsible for the lack of fractionation effects seen with MC, relative to other alkylating agents (37).

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Limited Sampling Model for Vinblastine Pharmacokinetics[1,2]

Mark J. Ratain* and Nicholas J. Vogelzang[3,4]

A limited sampling model was developed for vinblastine to estimate the total area under the concentration time curve (AUC) using only two timepoints. Detailed pharmacokinetic analysis (16 timepoints) was performed in 30 patients treated with a small bolus dose (3 mg/m^2) of vinblastine. A model for the total AUC was developed by multiple linear regression, using the first 15 patients as the training data set: AUC = 38.0 C_{10} + 73.8 C_{36} − 12.9, where C_{10} and C_{36} represent the serum vinblastine concentration at 10 hours and 36 hours, respectively ($r = 0.99$, $P < 0.0001$). The model was validated on the other 15 patients, the test data set ($r = 0.94$, $P < 0.0001$), with a mean predictive error of 13%. Limited sampling models may facilitate large-scale pharmacodynamic studies of new anticancer drugs, in order to relate the estimated AUC to toxicity and/or response without the need for detailed pharmacokinetic analysis. [Cancer Treat Rep 71:935-939, 1987]

Since most anticancer drugs have a very narrow therapeutic index, it is important to maximize our understanding of anticancer drug pharmacology, and eventually devise more rational dosing regimens. Although pharmacokinetic studies are generally performed in conjunction with phase I clinical trials, the pharmacodynamic results are seldom correlated with the observed toxicity. As a result, our knowledge of anticancer drug pharmacodynamics is limited, with only a few published studies demonstrating significant pharmacodynamic relationships (1).

Pharmacodynamic studies of anticancer drugs are difficult to perform for a number of reasons. Anticancer drugs are usually given in combination; thus, it is difficult to attribute pharmacological effects to a single drug. There is also often a delay of 1-6 weeks between drug administration and the maximal toxic effect, thus necessitating infrequent and intermittent dosing, and careful patient observation over prolonged periods of time.

Several recent studies by Egorin et al (2-4) have demonstrated that the area under the concentration time curve (AUC) can be quantitatively related to the dose-limiting myelosuppression for some drugs. This relationship is likely to be clinically useful, as it is consistent with two previously published models for cell-cycle nonspecific agents, based on in vitro experiments (5,6). However, it is not very feasible to do detailed pharmacokinetic profiles on large numbers of patients to precisely determine the AUC, for purposes of defining pharmacodynamic relationships.

Rather, it would be desirable to develop a limited sampling model to estimate the total AUC, based on analysis of serum concentrations at only two timepoints. This concept is similar to the current standards of aminoglycoside dosing, using only peak and nadir blood levels (7).

MATERIAL AND METHODS

Patients

The 30 patients studied were part of a phase I trial of vinblastine by prolonged continuous infusion (8,9). All patients had histologic proof of malignant disease refractory to standard therapy, and acceptable bone marrow function (wbc count > 3000/μl and platelet count > 75,000/μl), hepatic function (bilirubin < 2.0 mg/dl), and renal function (creatinine < 2.0 mg/dl). Written

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[3] Lynn Sage Clinical Pharmacology Laboratory for Cancer Research, Department of Medicine, and the Committee on Clinical Pharmacology, University of Chicago Pritzker School of Medicine and Michael Reese Hospital and Medical Center, IL.

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TABLE 1.—Summary of interpatient variability at each timepoint

	Vinblastine concentration (ng/ml) at time—															
	5 mins	10 mins	20 mins	30 mins	45 mins	1 hr	1.5 hrs	2 hrs	4 hrs	7 hrs	10 hrs	12 hrs	24 hrs	36 hrs	48 hrs	72 hrs
Mean	51.2	26.1	17.5	13.1	11.1	9.2	7.1	6.0	3.4	2.5	1.76	1.52	0.95	0.70	0.51	0.36
SD	27.7	12.5	8.1	4.4	3.9	3.5	2.8	2.7	1.8	1.2	0.76	0.60	0.39	0.35	0.25	0.20
CV (%)*	54.1	47.8	46.2	33.6	34.9	37.4	39.5	45.2	51.6	48.4	43.3	39.2	41.1	49.9	49.5	54.4

*CV = coefficient of variation.

informed consent was obtained in accordance with federal and institutional guidelines.

Pharmacokinetic Studies

Patients were treated with a small iv bolus dose (3 mg/m^2) of vinblastine, and serum samples were obtained at 16 timepoints following drug administration (5, 10, 20, 30, 45, 60, and 90 mins; 2, 4, 7, 10, 12, 24, 36, 48, and 72 hrs). The serum vinblastine concentration was determined by radioimmunoassay, as described previously (8).

All pharmacokinetic and statistical analysis was performed on a Professional 350 microcomputer (Digital Equipment Corp, Waltham, MA) using the RS/1 software package (Bolt Beranek and Newman, Cambridge, MA). The concentration versus time data were sequentially fitted to both three-compartment and two-compartment open models using a weighting of the reciprocal of the square of the concentration. The best model was selected, as previously described (9). The AUC and area under the moment curve (AUMC) were calculated by the log trapezoidal method, and extrapolated from zero to infinity according to the model derived above. The mean residence time (MRT) was calculated as: AUMC/AUC (10). The fraction of the AUC in the terminal phase was calculated as:

$$\frac{(B/\beta)}{AUC},$$

where B and β are the coefficient and elimination constants, respectively, of the terminal phase.

Statistical Analysis

The first 15 patients (as chronologically entered) constituted the training data set, and the subsequent 15 patients formed the test data set. Using the training data set, a separate univariate analysis was performed for each timepoint correlating the respective vinblastine concentration with the AUC. Subsequently, stepwise forward multiple regression analysis was used to generate the best model with two or fewer variables for the training data set. The F test was used to select the optimal model. The model was validated using the test data set, correlating the estimated and actual AUC.

RESULTS

Interpatient Variability

The interpatient variability in vinblastine pharmacokinetics for the first 24 patients was described in detail in a prior report (9). In analyzing all 30 patients, there was significant interpatient variability in the vinblastine concentration at all timepoints (table 1). The least variability occurred from 30 minutes to 60 minutes, with the maximal variability at 5 minutes and 72 hours. The AUC averaged 109 ± 51 ng hr/ml (range, 45–330), and the MRT averaged 31.7 ± 11.3 hours (range, 15.1–70.9).

Model Development

The vinblastine concentrations at each timepoint were correlated with the total AUC, using the training set (table 2). There was only a modest correlation at early timepoints (5–20 mins), but the correlation coefficient was > 0.85 for all timepoints at 1 hour or later. The two single timepoints with the best univariate correlation were 10 hours and 36 hours, each with a correlation coefficient of 0.97 ($P < 0.0001$).

For the multiple regression analysis only 13 timepoints were used, eliminating the three variables with incomplete data sets, since the correlation coefficient in each case was modest. Not surprisingly, the best model

TABLE 2.—Correlation of the serum vinblastine concentration at each timepoint with the total AUC for the training data set

Time	n	r
5 mins	12	0.22
10 mins	13	0.48
20 mins	15	0.45
30 mins	14	0.68
45 mins	15	0.58
60 mins	15	0.86
90 mins	15	0.88
2 hrs	15	0.95
4 hrs	15	0.88
7 hrs	15	0.94
10 hrs	15	0.97
12 hrs	15	0.90
24 hrs	15	0.87
36 hrs	15	0.97
48 hrs	15	0.91
72 hrs	15	0.92

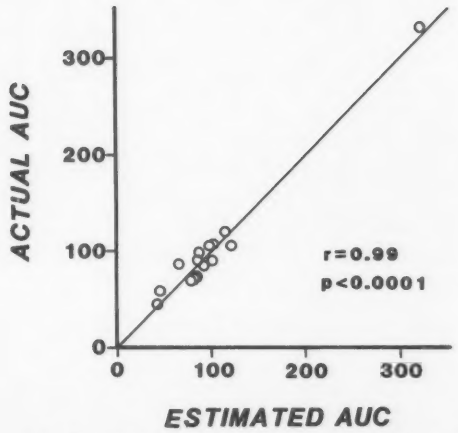

FIGURE 1.—Correlation of the estimated (from the model) and actual AUC (ng hr/ml) for the training data set. The solid line is the regression line, and is equivalent to the line of identity.

using only two variables incorporated the two best variables from the univariate analysis: AUC = 38.0 C_{10} + 73.8 C_{36} − 12.9, where C_{10} and C_{36} represent the serum vinblastine concentrations at 10 and 36 hours, respectively. This model was significantly better than the best univariate model, using only C_{10} ($P < 0.001$), despite the high correlation (r = 0.92) between C_{10} and C_{36}. Testing of the model on the training data set from which it was derived shows an excellent fit (fig 1).

Validation of the Model

An important step in the development of a mathematical model is validation on a test data set, distinct from the training data set. The second group of 15 patients formed the test data set for this analysis. The model was highly predictive in estimating the total AUC for this data set as well, although the correlation coefficient was slightly less than that for the training data set (fig 2).

Additional Model

Although our stated objective was to use two or fewer timepoints, an additional model was developed which included the serum vinblastine concentration at 90 minutes ($C_{1.5}$):

$$AUC = 5.00\ C_{1.5} + 19.0\ C_{10} + 81.9\ C_{36} - 18.0.$$

This model was significantly better than the bivariate model ($P < 0.005$), but only marginally increased the multiple r for the training data set from 0.988 to 0.994. When validated on the test data set, the correlation coefficient increased slightly from 0.94 to 0.95.

Analysis of the Model

Although there was an excellent fit of the model to the test data set (r = 0.94), there was a trend towards underestimating the actual AUC, occurring in 11 of 15 patients. The predictive error for the test data set averaged 13% (range, 1%–33%), acceptable given the 27% coefficient of variation (116 ± 31 ng hr/ml) and 2.5-fold range (71–177 ng hr/ml) in the actual AUC. However, there were no patients in the test data set with a very high AUC (> 300 ng hr/ml), as occurred in one patient in the training data set, making it impossible to validate the model in patients with markedly decreased vinblastine clearance.

Since both of the timepoints in the model were relatively late, it was considered that the model might only be useful for those patients with a high fraction of the total AUC in the terminal phase. This fraction ranged from 0.42 to 0.80 (mean, 0.68 ± 0.09), but was not significantly correlated with the predictive error. However, there was a borderline significant correlation between the MRT and the predictive error, as shown in figure 3. A similar relationship was not found for the training data set. This suggests that this particular model might be less useful for patients with a higher MRT (delayed clearance).

DISCUSSION

In this report, we have demonstrated that a limited sampling model can be developed for vinblastine, which estimates the total AUC from the serum vinblastine concentration at only two timepoints (10 hrs and 36 hrs). The two timepoints could not have been chosen from simple analysis of the coefficient of variation of the individual vinblastine concentrations (table 1) but were only revealed using stepwise multiple regression. A model was also developed using three timepoints which was statistically optimal, but not substantially better in its predictive value than the two-point model, and thus would not be recommended for practical reasons. A

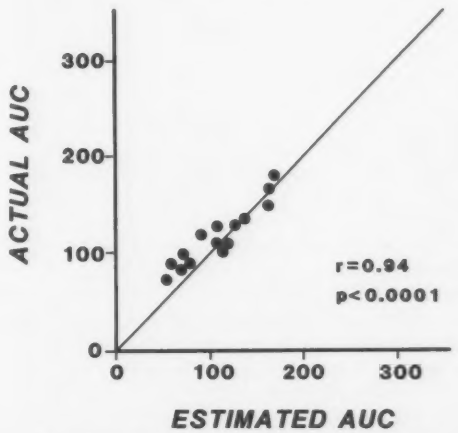

FIGURE 2.—Correlation of the estimated (from the model) and actual AUC (ng hr/ml) for the test data set: actual AUC = 0.76 (estimated AUC) + 33.4. The solid line is the line of identity.

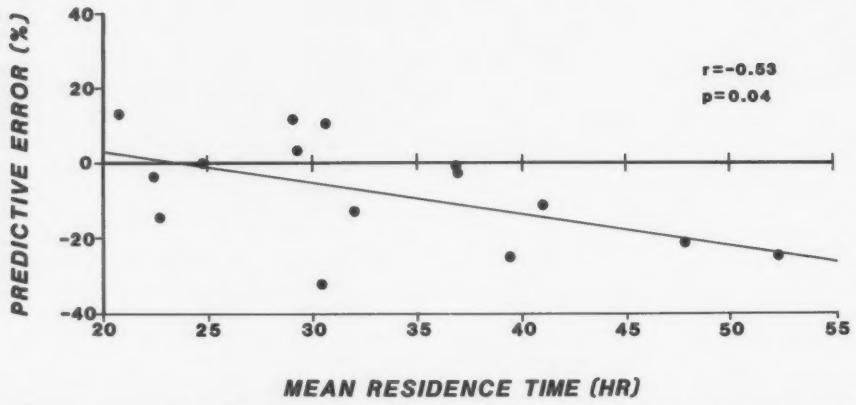

FIGURE 3.—Correlation of the MRT and predictive error (100 × [estimated AUC − actual AUC]/actual AUC) for the test data set.

limited sampling model has also been developed for thiotepa in 11 patients treated at four different dose levels (with normalization for dose) using three timepoints (45 mins, 2 hrs, and 4 hrs) (11). Therefore, it may be possible to develop limited sampling models for many drugs, which would enable the development of pharmacodynamic studies which require determination of the total AUC.

Certain characteristics of a drug would facilitate the development of a limited sampling model. The ideal drug must have linear pharmacokinetics, if the data available for development of the model are at multiple dose levels (and thus require normalization for dose). In addition, the ideal drug for initial studies would be a cell-cycle nonspecific agent, whose dose-limiting toxicity is myelosuppression, such as an intercalating agent. Theoretically there is a log-linear relationship for this class of drugs between the total AUC and the survival fraction of the wbc count or platelet count—the ratio of the nadir count to the pretreatment count (12). In contrast, the pharmacodynamics of phase-specific agents, such as methotrexate, may require much more complicated mathematical models (13). Thus, this specific model for vinblastine (an M-phase specific drug) has not been utilized, but is only presented as an example.

A limited sampling model may not be possible for all drugs. Drugs which have marked interpatient variability in the shape of the concentration-time curves may not allow for accurate estimation using only two timepoints. This was not a problem with vinblastine, as the fraction of the total AUC in the terminal phase had relatively little interpatient variability (0.68 ± 0.09), as compared to the much greater interpatient variability in other pharmacokinetic parameters (9).

It is also important to emphasize that there may be multiple useful limited sampling models which can be derived from a set of pharmacokinetic data, especially if the data for each patient can be fit to a pharmacokinetic model. In this case, concentrations at timepoints not actually sampled could be utilized in model development, possibly resulting in a superior model. Thus, future pharmacodynamic studies of vinblastine using a limited sampling scheme need not adhere to timepoints described in this paper if an alternate optimal sampling scheme has been explored.

It will be useful to develop limited sampling models for new drugs as phase I trials are completed, so that when validated, the models could be prospectively employed in phase II clinical trials to answer pharmacodynamic questions regarding toxicity and/or response. It is not claimed that the limited sampling model provides an exact estimate of the actual AUC, since the predictive error ranged up to 33%. However, the use of limited sampling models may provide a better understanding of interpatient pharmacodynamic variability, and make feasible a greater number of pharmacodynamic studies, if elaborate pharmacokinetic sampling is not necessary. It is conceptually simple in comparison to Bayesian models described by other investigators (14,15). Hopefully, the limited sampling model will lead to a better understanding of anticancer pharmacodynamics and optimization of dosing.

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Cyclophosphamide, Doxorubicin, and Vincristine in Etoposide- and Cisplatin-Resistant Small Cell Lung Cancer[1,2]

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For two chemotherapy regimens to be truly non-cross-resistant, each should be active as first-line therapy and also as second-line therapy. The effectiveness of both cyclophosphamide, doxorubicin, and vincristine (CAV) and etoposide and cisplatin (VPP) in previously untreated patients with small cell lung cancer has been well-documented. Also, VPP has caused tumor regression in up to 50% of patients when used as second-line therapy after CAV. The effectiveness of CAV after progression or relapse after VPP has not been documented. We identified 29 patients who received CAV after their tumors failed to respond or relapsed after VPP or etoposide and carboplatin (VPC). There were 21 male and eight female patients (median age, 57 years; range, 30–79). Thirteen patients were treated following failure to respond to VPP or VPC and 16 at the time of relapse. Eight patients had limited disease and 21 had extensive disease. Metastatic sites included liver (11 patients), bone (ten), lymph nodes (seven), bone marrow (three), brain (four), and contralateral lung (one). There were three complete responses (durations of 16, 22, and 26 weeks) and five partial responses (duration, 8+ to 36 weeks). Three patients had stable disease and 18 patients had disease progression while receiving treatment. The median survival of the entire group was 15 weeks. Responding patients had a median survival of 34 weeks (range, 8+ to 72+) and stable and nonresponding patients had a survival of only 9 weeks (range, 2–38). A granulocyte count nadir $< 500 \times 10^9$/L was seen after 7.9% of evaluable treatment cycles and a platelet count nadir $< 50{,}000 \times 10^9$/L occurred after only 5.3% of cycles. Five patients required transfusion for anemia, and two patients required dose reduction of vincristine for peripheral neuropathy. This study demonstrates that CAV has limited activity following failure to respond to VPP. [Cancer Treat Rep 71:941–944, 1987]

Small cell lung cancer (SCLC) accounts for 20%–25% of all bronchogenic carcinomas and is distinguished from other pulmonary neoplasms by its exquisite sensitivity to chemotherapy. The introduction of multiple-drug combination chemotherapy for SCLC in the early 1970s resulted in a significantly higher percentage of responding patients and improved overall survival (1). Although response rates $\geq$ 80% are achieved regularly in both limited- and extensive-disease patients with several drug combinations (2), relapse occurs early and $<$ 20% of the patients with limited disease are alive $>$ 2 years.

In an attempt to increase response and prolong survival, recent clinical trials have focused on the alternation of non-cross-resistant drug combinations (2). For two chemotherapeutic regimens to be truly non-cross-resistant, each should be active both as first-line and as second-line therapy. The combination consisting of cyclophosphamide, doxorubicin (Adria Laboratories, Columbus, OH), and vincristine (CAV) has been shown to be an effective first-line regimen for SCLC (3). More recently, the combination of etoposide and cisplatin (VPP) has demonstrated equal effectiveness when used as first-line therapy (4) and has also been shown to be an effective treatment for relapse of SCLC treated initially with CAV (5). However, the efficacy of CAV following VPP has not been demonstrated to date.

In an attempt to assess whether these two regimens are non-cross-resistant, we attempted to treat all patients with CAV after failure to respond or relapse after initial treatment with VPP and these patients form the basis of this report.

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PATIENTS AND METHODS

The records of the Toronto General Hospital and the Princess Margaret Hospital were reviewed and 73 patients with SCLC who had undergone initial induction therapy with VPP between 1982 and 1986 were identified, as well as nine patients who had been treated with etoposide and carboplatin (VPC) in a National Cancer Institute of Canada trial. Twenty-nine of these 82 patients who were refractory to treatment or who had relapsed after an initial response were subsequently treated with CAV. Our retrospective analysis of their response to this second-line treatment forms the basis of this report.

Baseline investigation included a complete history and physical examination; cbc with differential and wbc and platelet counts; serum electrolytes; BUN; creatinine; liver function tests; chest x-ray; bone, liver, and brain scans; and a bone marrow aspiration and trephine biopsy. Before each treatment, assessment consisted of physical examination, chest x-ray, cbc, and chemistry. Scans were repeated at the completion of treatment or earlier, if necessary, to document response.

Chemotherapy for all patients consisted of cyclophosphamide (1000 mg/m^2), doxorubicin (50 mg/m^2), and vincristine (2 mg), all given iv on Day 1 and repeated every 3 weeks in the absence of toxicity. Doses were not reduced because of prior treatment, although two patients had a 25% reduction of doxorubicin because of pre-existing cardiac disease. Patients were required to complete two courses of CAV to be considered evaluable unless there was definite evidence of disease progression after the first course. Standard response criteria were employed, and response duration was measured from the date of first treatment with CAV to the date of disease progression. Survival time was measured from the time of first treatment with CAV to the date of death.

RESULTS

Characteristics of the 29 patients are shown in table 1. There were 21 male and eight female patients, with a median age of 57 years (range, 30–79). Only nine patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. Eight patients had limited disease, while 21 had extensive disease. Of the 21 patients with extensive disease, 11 had liver involvement, ten had bone involvement, seven had extrathoracic or contralateral supraclavicular lymph node involvement, four had brain metastases, three had bone marrow involvement, and one had bilateral lung lesions.

Sixteen of the 29 patients had demonstrated a response to initial treatment with VPP or VPC. Six patients had achieved complete response and ten patients had achieved partial response. Six patients had had stable disease and seven had demonstrated disease progression while still receiving chemotherapy. The median interval from last treatment with VPP or VPC to the start of CAV was 4 weeks (range, 3–26).

Four patients had also received mitoxantrone as part of a phase II trial of this drug in patients who had not received prior anthracycline therapy. Two patients had had one course of treatment, one patient had had two courses, and one patient had received three courses. No patient had demonstrated a response to mitoxantrone.

Response and Survival

Three patients achieved complete response to treatment with CAV. This occurred in two of the eight patients with limited disease (duration, 22 and 26 weeks) and in one of the 21 patients with extensive disease (duration, 68 weeks). Three patients with limited disease and two with extensive disease achieved partial response (duration, 8+–36 weeks). Three patients had

TABLE 1.—Characteristics and response of 18 patients treated with CAV after VPP or VPC*

	Limited disease	Extensive disease	Total
No. of patients	8	21	29
Median age in yrs (range)	53 (47–72)	57 (30–79)	57 (30–79)
Male:female ratio	4:4	17:4	21:8
Performance status (ECOG)			
0	0	1	1
1	4	4	8
2	4	14	18
3	0	2	2
Prior response to VPP or VPC			
Complete	2	4	6
Partial	3	7	10
No response	3	10	13
Response to CAV (duration in wks)			
Complete	2 (22, 26)	1 (68)	3
Partial	3 (8+, 32, 36)	2 (8, 12)	5
Stable disease	1	2	3
Disease progression	2	16	18

*Unless otherwise specified, values = No. of patients.

stable disease and 18 patients had definite progression of disease while receiving treatment. One patient had marked improvement, with almost complete clearing of a pleural effusion and re-expansion of lung. Because this patient did not have measurable disease, he was classified as stable rather than partial response.

Sixteen patients who had demonstrated prior response to VPP or VPC were treated at the time of relapse. Of these patients, one achieved complete response, three achieved partial response, and one had significant improvement. Thirteen patients were treated with CAV after failing to respond or progressing while receiving VPP. Two of these patients achieved complete response and two achieved partial response.

No patient responded who had been treated initially with carboplatin. Of the four patients who had received mitoxantrone, one achieved compete remission, one had improvement and stable disease for 27 weeks, and two progressed.

The overall median survival of the 29 treated patients was 15 weeks. Those patients who achieved complete or partial remission had a median survival of 34 weeks (range, 8+ to 72+). Patients with stable and progressive disease had a median survival of only 9 weeks (range, 2-38).

Toxicity

Sixty-three cycles of CAV were administered. The median number of treatment cycles was two (range, one to seven). Twelve patients received only one treatment cycle. Treatment was stopped in all patients because of disease progression rather than toxicity, although one patient died with pneumonia at 2 weeks. Nausea and vomiting was present to some degree for all patients, but treatment was not stopped in any patient because of this toxic effect. Alopecia could not be assessed because of prior chemotherapy and radiotherapy in almost all patients. A granulocyte count nadir $< 500 \times 10^9$/L was seen after only 7.9% of evaluable treatment cycles, and a platelet count nadir $< 50{,}000 \times 10^9$/L was seen after only 5.3% of evaluable treatment cycles. There were no episodes of neutropenia-associated sepsis or bleeding, and only two patients required a 25% dose reduction for neutropenia. Five patients developed anemia < 100 g/L and required transfusion. Two patients developed peripheral neuropathy which required discontinuation of vincristine.

DISCUSSION

Although complete and partial response rates $\leq 80\%$ are achieved regularly with current combination chemotherapy for SCLC, response duration is short. Long-term survival is $< 20\%$ for patients with limited disease who achieve complete remission and rare for patients with extensive disease (6). The addition of mediastinal irradiation and prophylactic cranial irradiation has resulted in reduced rates of failure at the primary site and in the central nervous system, respectively, but has had no significant impact on overall survival (7). For that reason, recent attempts to improve the treatment for SCLC have focused on modifications of drug dosage and scheduling, such as early and late intensification and the use of alternating non-cross-resistant regimens.

There is no question that response and survival are superior in SCLC when multiple drug combinations are employed, although collected results from the literature suggest that there is little benefit to the *simultaneous* administration of more than three or four drugs, since the dose intensity of each agent must be sacrificed to prevent unacceptable toxicity (8). The desire to incorporate as many active agents as possible in the early stages of treatment led to the intuitive development of several trials of alternating drug combinations. More recently, this approach has been supported by the mathematical model of Goldie et al (9). Their somatic mutation theory suggests that the efficacy of treatment will be enhanced by the earliest possible introduction and the most rapid alternation of all active agents in a therapeutic program. This has been supported by reports of improved survival for patients with advanced Hodgkin's disease treated with mechlorethamine, vincristine, procarbazine, and prednisone alternating with doxorubicin, bleomycin, vincristine, and dacarbazine (10). Unfortunately though, in SCLC only modest clinical benefit has been seen in most clinical trials of alternating chemotherapy combinations (2,11,12).

The somatic mutation model has strict requirements that must be met before alternating sequence protocols can be expected to produce superior results (9). It is critical that the drug regimens employed be non-cross-resistant and quantitatively equivalent. Although many drug combinations have demonstrated approximately equal activity when used as initial therapy for SCLC, most have not been shown to be truly non-cross-resistant by demonstrating activity in patients who are resistant to other first-line regimens.

It has been suggested that CAV and VPP are non-cross-resistant (5), and this has led to the development of several prospective trials incorporating these regimens in either alternating or sequential fashion (12-14). Certainly, both CAV and VPP have been shown to have approximately equivalent activity when used as first-line therapy (3-5), and VPP has also been shown to be an effective treatment for relapse in SCLC (5).

In an attempt to determine if there is bidirectional non-cross-resistance between these two combinations, we undertook to treat all patients with CAV if they failed to respond or relapsed after VPP. It is of importance to note that of 82 patients treated initially with VPP, only 29 (34.3%) were felt to be suitable for second-line therapy at the time of disease progression. Furthermore, even within this select subgroup, only nine patients continued to have an ECOG performance status of 0 or 1, whereas at initial presentation, the majority of patients (19 of 29) were in this good performance status subgroup. It was also noted that 11 patients presented initially with limited disease and 18 had extensive dis-

ease; however, at the time of treatment with CAV there were only eight remaining patients with limited disease and 21 had distant metastases. These observations illustrate the differences in patient characteristics that occur during treatment with first-line therapy in SCLC and serve to emphasize the difficulty which may be expected in studies designed to document non-cross-resistance or in phase II studies hoping to find new agents with activity in SCLC.

In our study, response was seen in eight of 29 patients (27.6%). Sixteen of our patients were treated with CAV at the time of relapse following an initial response to VPP. One of these patients achieved complete remission and three achieved partial remission with CAV. Thirteen additional patients were treated with CAV after progression on VPP, and of interest was the observation that two of these patients achieved complete response and two had partial response. It is the response of these patients which suggests that CAV and VPP may be non-cross-resistant combinations. On the other hand, Scher et al (15) observed no responses among 40 patients treated with CAV following intensive induction with VPP and radiotherapy. Our results are also superior to those of Sculier et al (16), who reported only an 8% partial response rate and a 30% stable disease rate in patients treated with CAV after etoposide and vindesine with or without cisplatin. It is of interest, though, that partial response was observed in three patients who had failed to respond to their initial therapy. The slightly higher response rate seen in our study may be due to patient selection since only patients with adequate performance status were offered second-line treatment.

In summary, we have identified 29 patients who received treatment with CAV following initial treatment with VPP or VPC. Response was seen in 27.6% of these patients, including four of 13 patients who were progressing on VPP or VPC. This small study suggests that these two combinations may be non-cross-resistant in some clinical situations, but larger series would be required to determine more precisely the frequency of this occurrence. It would appear, however, that VPP is more active as a second-line therapy when relapses occur after CAV than is CAV when used for relapse following VPP.

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Phase I Trial of Combinations of Recombinant Interferons β_{ser} and γ in Patients With Advanced Malignancy[1,2]

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Based upon in vitro and in vivo synergistic activity of Type I and Type II interferons (IFNs) in preclinical in vitro and in vivo studies, we initiated a phase I trial evaluating the doses, safety, and pharmacokinetics of combinations of recombinant DNA-produced human IFN-β_{ser} and IFN-γ in 27 patients with cancer. Twenty-four patients were treated with a 2-hour infusion of IFN-γ, followed by a 10-minute iv injection of IFN-β_{ser}, three times a week. Patients were entered on fixed dose levels of 1×10^6, 3×10^6, 10×10^6, 30×10^6, and 100×10^6 units of each IFN. In addition, three patients were treated at the highest dose level with a 10-minute iv infusion of IFN-γ and a 10-minute iv infusion of IFN-β_{ser}. The maximally tolerated dose when administered by this schedule for ≥ 4 weeks was 30×10^6 units of each IFN. Dose-limiting side effects at doses of 100×10^6 units of each IFN consisted of fatigue, nausea, vomiting, anorexia, paralytic ileus, and neutropenia. The most common side effects at the three highest dose levels were fever, rigors often requiring parenteral meperidine, and constitutional symptoms. Reversible elevations in SGOT and LDH were also noted. Serum IFN levels were dose related, with peak titers occurring immediately after IFN administration. One patient with a nodular mixed lymphoma had a partial response which has been sustained for over 1 year. We conclude that combinations of IFN-β_{ser} and IFN-γ can be safely administered on a chronic basis without enhanced or cumulative toxic effects.
[Cancer Treat Rep 71:945–952, 1987]

Interferons (IFNs) are prototypic lymphokines with a broad range of antiviral, immunomodulatory, and antiproliferative effects. Three major classes have been defined. Human IFNs α and β (Type I IFNs) are 166 amino acids in length; a comparison of the sequences of IFNs α and β demonstrates a 29% homology of amino acids (1–4). IFN-γ (Type II) is 143 amino acids in length, has a distinct amino acid sequence homology from IFN Type I, and differs from Type I IFNs in antigenic and biochemical properties (5). Unique biological properties of partially purified IFN-γ, when compared to IFN-α and IFN-β, include differences in receptor binding (6,7), differences in ganglioside affinity (8), more potent antiviral (9) and antiproliferative activity (8,10,11) in certain cell lines, and the induction of unique polypeptides (12). Furthermore, immunomodulatory effects of IFN-γ include macrophage activation (13,14), enhancement of Class I and II major histocompatibility antigen expression (15,16), stimulation of interleukin II receptors (17), and enhancement of tumor necrosis factor receptor expression (18–20).

Physicochemical and biological differences between IFNs have generated interest in their combined in vivo and in vitro effects. Early studies with impure preparations of murine Type I and Type II IFNs demonstrated synergistic antitumor activity (21–23). Subsequent studies with purified preparations of human IFN have confirmed a similar potentiation in human tumors or transformed cell lines, including transformed human amnion WISH cells (24), melanoma (25,26), histiocytic lymphoma (27), fibrosarcoma, colon, transitional cell, renal, and bronchogenic carcinoma cells (28). Synergy has been observed in tumors resistant to the antiproliferative effects of one or both IFNs (28).

Clinical investigations will be necessary to determine whether the combination of Type I and Type II IFNs also results in enhanced efficacy in the treatment of human neoplasms. This study reports the results of a

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phase I trial designed to determine the pharmacokinetics, tolerance, and maximal tolerated dose (MTD) of combinations of IFN-β_{ser} and IFN-γ, and to identify any enhanced or unexpected toxicity associated with this combination of lymphokines. The results from this trial have formed the basis for subsequent studies investigating the effects of combinations of IFN-β_{ser} and IFN-γ on immunological parameters and biological responses.

MATERIALS AND METHODS

Interferons

IFN-γ was provided by Biogen, Cambridge, MA, with a specific antiviral activity of 2×10^7 units/mg of protein. IFN-β_{ser}, a synthetic mutant protein with a substitution of serine for cysteine at amino acid position 17 and a specific activity of 1×10^8 units/mg (29), was provided by the Cetus Corporation/Triton Biosciences Interferon Program, Alameda, CA.

Patient Selection

This study was open to patients with histologically confirmed malignancies for which no standard therapy was known to be effective. Eligibility criteria included an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, an estimated life expectancy of at least 3 months, and adequate hematologic (granulocyte count $\geq 2000/mm^3$, platelet count $\geq 100{,}000/mm^3$, hematocrit $\geq 30\%$ or hemoglobin ≥ 10 g/dl), hepatic (bilirubin ≤ 1.4 mg/dl; SGOT $\leq$ twice normal), and renal function (creatinine ≤ 1.5 mg/dl; BUN ≤ 25 mg/dl). Patients could not have received cytotoxic chemotherapy for at least 3 weeks before initiation of IFN (6 weeks for mitomycin or nitrosoureas), radiation therapy for at least 2 weeks, or IFN-α or other immunotherapy for at least 4 weeks. Patients with clinically significant heart disease, CNS involvement, penicillin allergy, or prior treatment with IFN-β_{ser} or IFN-γ were excluded. While on study, patients were not allowed aspirin, corticosteroids, or nonsteroidal anti-inflammatory drugs. All patients gave informed consent.

Study Design

Patients were given a 2-hour iv infusion of IFN-γ, followed immediately by a 10-minute iv injection of IFN-β_{ser}. A 2-hour infusion of IFN-γ was chosen based upon available phase I safety data at the time the study was initiated. The 2-hour infusion of IFN-γ was administered before IFN-β_{ser} in order to ensure that the levels of the first IFN would be maintained during the infusion of the second IFN. Treatments were administered three times per week, and were separated by no less than 46 hours. Patients were routinely premedicated with oral acetaminophen, and every 6 hours thereafter for a total of four doses. After safety of the 2-hour infusion of IFN-γ was confirmed, an additional three patients were treated with a 10-minute infusion of IFN-γ followed by the 10-minute infusion of IFN-β_{ser}.

A minimum of three patients were entered at each dose level (table 1). Patients were removed from study for unacceptable toxicity (as defined below), or for progressive disease following the first 4 weeks of therapy. Patients with progressive disease in the absence of life-threatening organ dysfunction and a decline in performance status by more than two ECOG levels were allowed to continue another 4 weeks at the discretion of the investigator. Escalation proceeded to the next dose level once at least two patients completed 4 weeks of treatment with acceptable toxicity.

All patients underwent a physical examination, chest roentgenogram, ECG, and measurement of indicator lesion before entry onto study. Performance status was recorded with each treatment during the first month. Vital signs were obtained pretreatment, 1, 2, 4, 6, 8, and 12 hours post-injection on Days 1 and 3 of the first cycle, and thereafter as clinically indicated. Hematological, hepatic, and renal functions were monitored with each treatment for the first 2 weeks and weekly thereafter. Tumor assessments, physical examinations, and chest roentgenograms were obtained monthly.

IFN doses were modified for moderate and severe toxic effects. Moderate symptomatology was defined as symptoms that required medication for relief, did not resolve with repeated IFN treatments, or resulted in a 5%–10% weight loss from baseline, or a drop in performance status to ECOG level 3. Severe symptomatology included symptoms that were inadequately relieved by medication or caused a $> 10\%$ weight loss, or a drop in performance status to level 4. Changes in hematologic, hepatic, and renal function that were considered a moderate toxicity included a granulocyte count $< 500/mm^3$; platelet count between 50,000 and $25{,}000/mm^3$; SGOT 5–10 $\times$ normal; bilirubin 1.5–2 $\times$ normal; and creatinine 2.1–4.0 mg/dl. Severe laboratory toxic effects were defined as a granulocyte count $< 250/mm^3$; platelet count $< 25{,}000/mm^3$; SGOT $> 10 \times$ normal; bilirubin $> 2 \times$ normal; and creatinine > 4.0 mg/dl.

IFN treatments were withheld for any moderate to severe toxicity until the parameter returned to less than

TABLE 1.—Schema: dose of each IFN administered 3 times weekly

Dose level	Dose ($\times 10^6$ units)	
	IFN-γ*,†	IFN-β_{ser}*,‡
1	1	1
2	3	3
3	10	10
4	30	30
5	100	100

*Routes of administration: IFN-γ, 2-hr iv infusion; IFN-β_{ser}, 10-min iv injection.

†1000 μg $\approx 15 \times 10^6$ units.

‡1000 μg $\approx 180 \times 10^6$ units.

a moderate toxicity. Therapy was then resumed with the dose of each IFN reduced by one dose level. Patients who developed moderate fatigue or anorexia had their IFN reduced by one dose level without interruption of therapy. Therapy was then withheld if the performance status or weight loss did not improve at the reduced dose level.

Dose-limiting toxicity was defined as any moderate to severe toxicity which required a dose reduction during the first month of therapy. Once a dose-limiting toxicity occurred, up to six patients were treated at that dose level. The MTD was defined as the dose at which no more than one of six patients developed dose-limiting toxicity.

Pharmacokinetic Studies

Serum samples were obtained immediately prior to the initiation of the IFN-γ infusion and at 5, 10, 15, 30, 60, and 120 minutes thereafter (immediately following the completion of the 2-hour infusion and prior to the IFN-β_{ser} injection). Additional samples were obtained 1, 5, 10, 15, 20, 30, 60, 120, 180, 240, and 360 minutes after the 10-minute IFN-β_{ser} injection was completed, and at 24 hours.

Because an enzyme-linked immunosorbent assay (ELISA) specific for IFN-β_{ser} was not available, total serum IFN levels were assayed by Cetus in a cytopathic effect (CPE) assay with human fibroblast GM2504E cells using vesicular stomatitis virus (VSV) as the challenge virus (30). In each assay an internal laboratory standard was included that had been calibrated against an international reference preparation of human fibroblast interferon (G023-902-527). All titers are reported in IU/ml and are means of three titration results.

Serum IFN-γ was determined at Biogen by a modified double-antibody sandwich ELISA. An internal laboratory standard was used to generate a standard curve which had been calibrated against an international standard for antiviral potency, Gg 23-901-530, to give values expressed in IU/ml. All values represent the means of four titration results.

Interferon Antibody Studies

Sera for antibody determination to IFN-β_{ser} and IFN-γ were obtained pretreatment and at monthly intervals. When possible, serum samples were also obtained 14 and 60 days following the last IFN treatment.

An ELISA was performed at Triton to test patient sera for the presence of IgM and IgG antibodies that bind to IFN-β_{ser}. Patient sera were screened at a 1:30 dilution and values were compared to results from a panel of sera obtained from six normal volunteers. Patient sera were titered if the screening test showed a value that was twofold greater than the highest value obtained from the normal sera. The detection limit for IgM was 8 μg/ml; the detection limit for IgG was 3 μg/ml. Neutralization titers were determined for samples with positive immunoglobulin titers. Sera were tested for the ability to neutralize the antiviral effect of IFN-β_{ser} in a modification of the CPE assay described above. Dilutions of serum were incubated with a constant concentration of IFN-β_{ser}, 2.5 IU/ml. The results were calculated by multiplying the dilution of serum that reduces 2.5 IU/ml to 1 IU/ml by the difference of these values, 1.5 IU/ml. The detection limit of the assay was 3–5 neutralizing units per ml (NU/ml).

Levels of neutralizing antibody to IFN-γ were determined at Biogen using a CPE assay with WISH cells and encephalomyocarditis virus (EMV) as the challenge virus. Dilutions of either patient sera or a murine monoclonal anti-IFN-γ antibody were incubated with 10 IU/ml of IFN-γ. Neutralizing antibody was determined by comparing sample wells with wells containing the mouse monoclonal antibody to IFN-γ.

RESULTS

Twenty-seven patients were entered on study (table 2). Six patients had therapy discontinued within the first month, three because of progressive disease. Of the others, one patient underwent a surgical procedure, and a third elected to discontinue treatment for personal reasons. The second patient had a cardiorespiratory arrest thought to be unrelated to the IFN. This event occurred 13 days after he had been entered on study, and 24 hours after his last treatment of IFNs. The patient subsequently had a cardiac catheterization which revealed triple vessel disease and he underwent coronary bypass surgery. Because many of these patients experienced clinical side effects, these six patients were included in the toxicity analysis. However, they were not considered evaluable for dose-limiting toxicity (table 3).

Side Effects

Subjective side effects were generally dose related and qualitatively similar to those associated with IFN

TABLE 2.—Patient characteristics ($n = 27$)

Characteristic	No. of patients
Median age in yrs (range): 57 (18–71)	
Sex	
Female	11
Male	16
Prior therapy	
Chemotherapy	13
Radiation therapy	9
Interferon therapy	2
Diagnosis	
Renal cell cancer	10
Melanoma	10
Lymphoma	2
Colon cancer	1
Breast cancer	2
Sarcoma	2

TABLE 3.—Treatment summary

Dose levels ($\times 10^6$ units of each IFN)	No. of patients	No. of weeks (range)	No. of patients experiencing dose-limiting toxicity/No. of patients evaluable for dose-limiting toxicity
1	7	1-25	0/3
3	3	8-28	0/3
10	8	1-25	1/7
30	3	4-60	0/3
100	6*	1-19	5/5

*3 patients received IFN-γ by 10-minute infusion.

(table 4). Side effects were not clinically significant at the two lowest dose levels. The most common side effects at the three highest dose levels were fever ($\geq$ 39°C) and severe chills. Rigors requiring parenteral meperidine were observed in two of three patients treated with the equivalent of 30×10^6 units of each IFN, and in all patients treated with 100×10^6 units. A tachyphylaxis developed to the rigors, so that within 2 weeks the chills were generally moderately well controlled with acetaminophen. Constitutional symptoms, including moderate myalgias, headache, and nausea and vomiting, were also experienced at the higher dose levels and also resolved with repeated treatments. Moderate hypotension ($\geq$ 10-mm drop in diastolic blood pressure) occurred at all dose levels but was not sustained with time. Two patients treated with 10×10^6 units of each IFN developed a $>$ 20-mm drop in diastolic blood pressure after their first treatment which was asymptomatic, resolved without iv hydration, and did not recur. No moderate or severe CNS toxicity was observed. One elderly patient developed transient confusion with fever and iv meperidine, and a second patient developed mild depression which did not require treatment.

The 100×10^6 unit dose level produced statistically significant elevations in SGOT ($P = 0.033$) and LDH ($P = 0.043$), and a decrease in the platelet count ($P = 0.047$), by paired t-test. However, clinically significant hematological toxicity was not observed except at the highest dose level (table 5). One patient at the third dose level experienced a transient drop in his neutrophil count to $< 1000/mm^3$ which resolved without interruption of therapy. A patient at the highest dose level required a dose reduction for a neutrophil count $< 500/mm^3$. No clinically significant thrombocytopenia was observed. Hepatic dysfunction was generally mild at the lower doses and not clinically significant. Elevations in SGOT and LDH were reversible (table 5). No significant alterations in renal function were seen.

Three patients treated with the original administration schedule (2-hour infusion of IFN-γ followed by a 10-minute injection of IFN-β_{ser}) developed dose-limiting toxicity which required a dose reduction. One patient treated with 10×10^6 units of each IFN developed moderate fatigue and weight loss. Both patients evaluable for dose-limiting toxicity at the 100×10^6 unit dose level required a dose reduction. One patient experienced moderate nausea, vomiting, and anorexia, which resulted in a $>$ 5% weight loss. The second patient developed moderate fatigue, a paralytic ileus, and an absolute neutrophil count of $< 500/mm^3$. In both patients, these symptoms subsequently resolved with interruption of therapy and did not return for at least 1 month at the reduced dose.

The initial treatment schedule was designed based upon available phase I safety data with 2-hour infusions of IFN-γ. Subsequent studies have confirmed the safety of a more rapid infusion (31). Because of patient inconvenience associated with a 2-hour infusion, and reports indicating that prolonged infusions were associated with enhanced toxicity (31), we treated an additional three patients at the 100×10^6 unit dose level with a 10-minute iv infusion of IFN-γ and a 10-minute iv injection of IFN-β_{ser}. All three of these patients required dose reductions because of severe fatigue and a decline in performance status. There was no difference in the severity of the rigors, myalgias, or nausea when compared to the three patients who had received the same dose of IFN-γ over 2 hours.

TABLE 4.—No. of patients experiencing side effects during the first month of treatment: severity of side effects by dose level*

Side effect	Dose of each IFN ($\times 10^6$ units)				
	1 ($n = 7$)	3 ($n = 3$)	10 ($n = 8$)	30 ($n = 3$)	100 ($n = 6$)
Chills	0/0	1/0	3/0	2/0	4/1
Fever	0/0	0/0	5/0	0/0	5/0
Hypotension	2/0	1/0	1/2	0/0	1/1
Fatigue	0/0	0/0	3/0	1/0	4/1
Myalgias	0/0	1/0	1/0	1/0	2/0
Nausea/vomiting	0/0	0/0	1/0	0/0	3/0
Anorexia	0/0	0/0	0/0	0/0	1/0
Weight loss	0/0	0/0	1/0	0/0	2/0
Paralytic ileus	0/0	0/0	0/0	0/0	0/1

*Values = No. of patients with moderate/severe side effects.

TABLE 5.—Hematologic and hepatic toxicity during the first month of therapy*

	Dose of each IFN ($\times 10^6$ units)				
	1 ($n = 7$)	3 ($n = 3$)	10 ($n = 8$)	30 ($n = 3$)	100 ($n = 6$)
Neutrophil count nadir (cells/mm^3)					
> 1500	6	3	7	3	2
1500–1000	1	0	0	0	3
999–500	0	0	1	0	0
< 500	0	0	0	0	1
Maximum SGOT (U/L)					
< 50	7	3	5	0	0
50–150	0	0	3	3	5
150–300	0	0	0	0	1
> 300	0	0	0	0	0

*Values = No. of patients.

Responses

One patient with a nodular, mixed, poorly differentiated lymphocytic histiocytic lymphoma had a partial response to 30×10^6 units of each IFN. This 42-year-old white female had presented 2 years earlier with a nodular poorly differentiated lymphoma. Following 2 months of treatment with IFN-β_{ser} and IFN-γ, her peripheral adenopathy disappeared and her pari-aortic adenopathy on computerized tomographic (CT) scan regressed to sizes of uncertain significance. The patient received an additional 10 months of combination IFN treatment and has been followed for > 6 months on no therapy, without change in her disease status.

Pharmacokinetics

Mean serum IFN-γ and total levels are plotted against time (fig 1A and 1B, respectively). Low doses (1×10^6–3×10^6 units) of IFN gave extremely low IFN titers (< 15 units), which were at or below the lower limits of sensitivity for the respective assays.

Serum for IFN-γ was sampled during the 2-hour infusion. Peak serum levels occurred at 1–2 hours during the infusion and increased with increasing IFN-γ dose (fig 1A). The mean peak level achieved at the 100×10^6 unit dose was 129 units. In two patients who received 100×10^6 units over 10 minutes, the mean peak level of 1600 units occurred at the completion of the infusion. In these two patients, IFN activity was still present in the serum at 6 hours.

Total serum IFN levels, measured after completion of the IFN-β_{ser} infusion were also dose dependent (fig 1B). Because an assay specific for IFN-β_{ser} was not available, the relative contribution of IFN-β_{ser} and IFN-γ to total antiviral activity could not be determined. No levels of IFN were measurable in serum following the infusions of 1×10^6 units or 3×10^6 units. The mean peak level at the 100×10^6 unit dose was > 3000 units. IFN was cleared rapidly from the circulation, but was still present at low levels 4–6 hours later.

No patients developed neutralizing antibodies to IFN-γ. Four patients developed detectable titers of IFN-β_{ser} immunoglobulin-binding antibodies ranging from 15–270 μg/ml. Three of the four had low levels of neutralizing activity to IFN-β_{ser} ($\leq$ 60 NU/ml).

DISCUSSION

Alpha interferon has documented antitumor activity in selected hematological and solid tumor malignancies such as hairy cell leukemia, non-Hodgkin's lymphoma, multiple myeloma, chronic myelogenous leukemia, Kaposi's sarcoma, melanoma, and renal cell carcinoma (32,33). However, as a single agent, alpha interferon has not made a clinical impact on the more common solid tumors such as lung, colon, breast, and prostate. One therapeutic strategy to increase overall efficacy is to integrate IFN into more standard forms of therapy. IFNs are currently being studied in conjunction with standard cytotoxic agents (34), irradiation (35), and hyperthermia (36), in a combined-modality approach to the treatment of cancer.

Recent investigations have begun to define a series of complex lymphokine interactions. In addition to potentiating antiproliferative activity (21–28), combinations of Type I and Type II IFN synergistically enhanced NK cell killing (37). IFN-γ has increased tumor necrosis factor (TNF) receptor number (18–20), potentiated TNF antiproliferative effect (38,39), and increased the expression of IL-2 receptors (17). Thus, combining lymphokines may be one way of augmenting clinical antitumor activity, providing that enhanced toxicity does not preclude meaningful clinical application.

We undertook a phase I trial to determine the doses, side effects, and safety of combinations of IFN-β_{ser} and IFN-γ. Combinations of IFN-β_{ser} and IFN-γ resulted in side effects qualitatively similar to those commonly associated with IFNs. Fever, chills, and malaise were common acute symptoms which generally abated with repeated treatments. Rigors were more severe at high doses than we would have expected based upon our previous experience with each IFN alone. Dose-limiting toxic effects consisted primarily of constitutional symptoms and usually consisted of fatigue and malaise. Hepatic toxicity and myelosuppression were observed at high doses. The only qualitatively unexpected toxicity

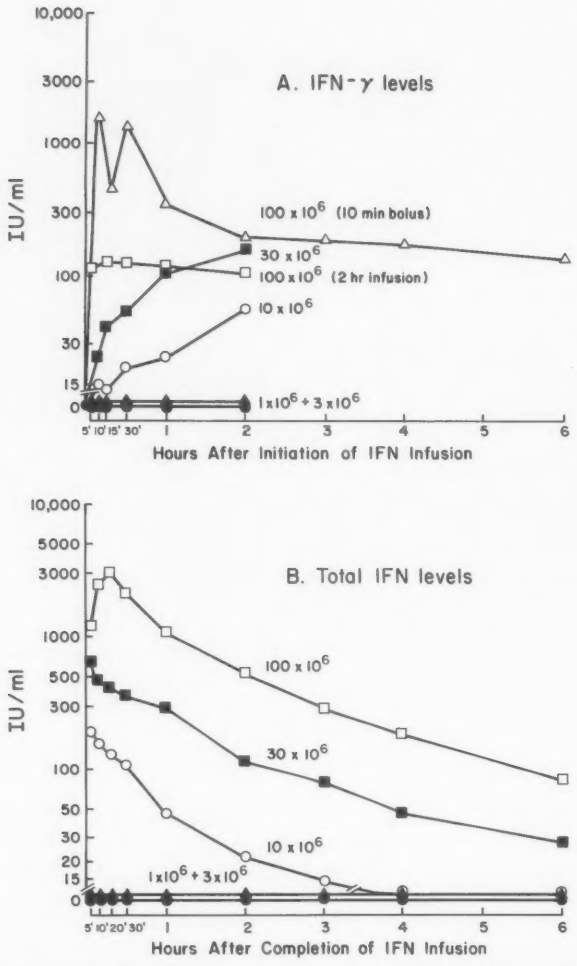

FIGURE 1.—Serum IFN-γ (fig 1A) and total IFN (fig 1B) levels during and after iv administration. Each point represents mean serum levels at that dose. No. of patients at each dose for whom serum IFN levels were available: 1×10^6 unit dose, $n = 7$; 3×10^6 unit dose, $n = 2$; 10×10^6 unit dose, $n = 8$; 30×10^6 unit dose, $n = 3$; 100×10^6 unit dose (IFN-γ, 2-hr infusion), $n = 2$; 100×10^6 unit dose (IFN-γ, 10-min iv bolus), $n = 2$.

was constipation, which in one patient resulted in a paralytic ileus. Severity of side effects was dose related. Continuing treatment for > 1 month generally resulted in a loss in performance status. However, in many patients this may also have been associated with disease progression. No clinically significant cumulative effects on bone marrow, renal, or hepatic function were observed. Preliminary results of a phase I trial with IFN-αA and IFN-γ suggest that more marked potentiation of systemic effects and cumulative toxicity may occur with these two IFN molecules (40).

Serum IFN levels were dose related. The 30×10^6 and 100×10^6 unit dose levels gave peak total serum IFN levels > 500 units which fell tenfold within 4 hours. A 2-hour infusion of 100×10^6 units of IFN-γ produced peak serum IFN-γ titers of < 500 units. The relationship between the antiproliferative and immunomodulatory properties of IFN and its antitumor effects is unclear. In vitro growth studies indicate that the antiproliferative effect of IFN is dose dependent, and that prolonged exposure times to IFN are required for greater growth inhibition (26,41). Thus, bolus injections or 2-hour infusions of high doses of IFNs may not produce serum levels comparable to the duration of exposure and concentrations of IFNs commonly used in the in vitro antiproliferative experiments upon which these

studies were based (26,28). However, clinical biological response modification may not be dose dependent (42,43), and may not require prolonged IFN infusions (30,43,44). Further in vitro antiproliferative and immunomodulatory studies are needed to test conditions defined by the MTD in this trial, and to correlate antitumor activity with peak IFN levels and the area under the curve.

In conclusion, a safe dose and schedule of combined IFN-β_{ser} and IFN-γ for phase II trials was defined as the equivalent of 30×10^6 units of each IFN three times per week. The MTDs of IFN-γ when given as a single agent have not yet been clearly defined when administered three times per week. However, 3000 $\mu g/m^2$ of IFN-γ (45×10^6 units/m^2), when given on a twice-a-week schedule, has been relatively well tolerated in phase II trials. The MTD of IFN-β_{ser} is 100×10^6 units when administered three times a week (45). Thus, the toxicity associated with combination treatment would appear to be additive. If a synergistic enhancement in antitumor activity results with only additive toxicity, a therapeutic benefit may be gained by combining these two interferons. Further systematically designed preclinical studies are needed, however, to develop an optimal therapeutic strategy. Optimal drug ratios and schedules may maximize the antitumor effects of combination IFN-β_{ser} and IFN-γ while minimizing toxicity. Additional phase I studies are currently under way to determine the effect of combination treatment on biological responses, and to define the optimal biologic dose and drug ratios.

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Continuous Sc Infusion of Narcotics for the Treatment of Cancer Pain: An Update[1]

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In 1973, Marks and Sachar (1) described inadequate analgesic treatment in a group of admitted medical patients. Since then, many excellent reviews have been published on improved therapy for the treatment of pain in different populations, including cancer patients. Most authors of cancer pain monographs have emphasized the advantages of regular administration of narcotics to maintain a stable blood level of the drugs and to prevent pain breakthrough, in contrast to taking the medication when pain is already present (2–6). However, many patients with cancer pain continue to receive narcotics as needed (7). When the patient requires parenteral narcotics, they are also administered as needed in many patients (7), although several authors have shown that both regular intermittent and continuous iv infusions of narcotics are safe and effective (8–12). Some of the reasons cited for the limited use of continuing parenteral narcotic therapy are physician's fear of narcotic overdose or tolerance, complications associated with the presence of an iv catheter for a long period of time, need for frequent nursing assessments of the infusion status, need for infusion pumps, or absence of peripheral veins in some patients (11–17). During recent years, the sc route has been suggested to be not only as effective as other methods of parenteral administration of narcotics, but also simpler and with less potential toxicity. If these advantages are confirmed, the sc route should replace other techniques for narcotic parenteral administration, therefore improving the quality of pain control in these patients.

The purpose of this paper is to review the literature on sc narcotic administration.

CONTINUOUS INFUSION OF NARCOTICS

Regular intermittent injections of narcotics can control cancer pain in most patients (2–4,8,9). However, because of the short duration of action of most narcotics, the injections need to be repeated, usually at least every 4 hours. Thus, this method becomes painful for the patient, time-consuming for nursing staff, and difficult to maintain in a home setting. A continuous iv infusion (CIVI) of narcotics provides a stable blood level of the drug, therefore reducing "peaks" associated with sedation or nausea and "valleys" associated with recurrent pain. The occurrence of toxicity soon after a dose of narcotics or pain breakthrough before the next dose has been defined as "bolus effect" (17). Patients with these problems are particularly good candidates for a CIVI.

Many articles provide evidence that a continuous administration of narcotics is safe and effective for the treatment of postoperative and cancer pain (10–17). Although there are no comparative studies between continuous infusion and intermittent injections in cancer patients, there are two such studies on postoperative pain. Rutter et al (13) studied 45 patients with postoperative pain who were randomized to receive a CIVI of morphine (Group A) versus regular im injections (Group B) versus im injections as needed (Group C). Pain control was significantly better in Group A than in Group C (Group B scored lower values than A but they were not significant). Patients in Group A also required significantly less morphine than those in Groups B and C. They concluded that morphine administered as CIVI was superior to the other regimens. Briggs et al (14) studied 42 patients who, after surgery, were randomized to receive morphine as a CIVI versus regular im injections. No difference was found in pain control or side effects. However, they concluded that the CIVI was the preferred route because of ease of administration, elimination of injections, and "possibly more even pain control" (although no evidence was provided in the paper for this last statement). Most studies have found CIVI to be very safe, with sedation and confusion as the most frequent side effects. In one series of 400 consecutive patients treated with CIVI of morphine for postoperative pain, there was not one episode of respiratory depression requiring narcotic reversal (14). In cancer patients, many uncontrolled reports suggested that a CIVI of narcotics is safe and effective (12–16). Portenoy (17) has recently completed an excellent review on CIVI in the treatment of cancer pain. Even though cancer patients receiving CIVI are usually in the terminal stage of their disease and, therefore, are rapidly deteri-

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orating, the toxicity of CIVI has always been found to be low, consisting mainly of sedation or confusion (17). However, this method is not infallible: Portenoy estimates that it was unsuccessful in one-third of the patients, mostly because of deafferentation pain, episodic pain, or patients with "total suffering" rather than nociceptic stimulus. These are three situations for which there is no effective treatment at the present time and CIVI is as ineffective as other analgesic methods for these patients.

USE OF THE SC ROUTE

Although most of the available evidence supports the view of CIVI of narcotics as a safe and effective technique, there are some problems with its use in cancer patients. Starting or maintaining an iv line in these patients can be very difficult because they have usually received multiple treatments with sclerosing agents and there are few available peripheral veins. Once the CIVI is started, patients may find the limited use of the arm where the iv line is inserted uncomfortable. In some cases, patients who are already receiving iv medications may need a second iv line because of incompatibility of narcotics with the other solutions (eg, total parenteral nutrition). If the iv cannula becomes displaced or blocked by a blood clot, a physician or a specially authorized nurse may be required to restart the infusion. Although the totally implantable central venous catheters such as a port-a-cath (18,19) could represent an improvement as compared to the use of a peripheral line, these catheters are expensive, have to be surgically implanted, and require considerable nursing expertise and patient teaching. Finally, home discharge of patients on a CIVI is expensive and difficult.

In 1979, Russell (20) described a method for sc administration of morphine to patients with advanced cancer. Since then, other authors have reported on the use of continuous sc infusions (CSCI) for the treatment of postoperative and cancer pain (21–31). Though most of the reports are based on a small number of patients, they strongly suggest that CSCI is safe and efficacious. In a consecutive series of 56 patients, we found that after 48 hours, 54 patients (96%) preferred CSCI to an equal dose of CIVI (42 patients) or intermittent injections (14 patients) (28). Twenty-five patients (45%) were discharged home for a median period of 18 days (range, 2–138). In Coyle's series, 13 of 15 patients (87%) had adequate pain control and were maintained on CSCI from 3 to 76 days. The remaining case reports have all suggested excellent pain control (20–23,26,27,29).

A major concern with CSCI of narcotics is irregular absorption because of uneven skin perfusion, with the potential for excessive absorption during periods of increased perfusion. Waldmann et al (32) have determined blood levels of morphine in nine patients receiving CSCI for postoperative pain and in four patients receiving CIVI at the same dose (0.05 mg/kg/hour). They found no difference between CSCI and CIVI after 6, 12, 18, and 24 hours of infusion. Mean blood level was 20 ± 7 ng/ml. Nahata et al (33) determined morphine blood levels in three children receiving CSCI at a rate of 0.03–0.06 mg/kg/hour for the treatment of terminal cancer pain and found a median blood level of 20 ng/ml. These two studies suggest that blood levels during CSCI of morphine are quite stable and do not seem subject to sudden changes.

In most cases, CSCI was started in the subclavicular region, anterior chest, or abdominal wall. Some authors recommended a change in the site of infusion every 2–3 days (24,30), while others suggested that the needle could stay in place for weeks (20,21). Because of the absence of prospective studies on the duration of the site, we recently conducted a prospective study in 45 patients receiving CSCI of narcotics (34). Once the CSCI was started, the site of infusion was not changed until signs or symptoms of intolerance developed (pain, redness, swelling, or leakage). Triceps skinfold was determined as an estimation of the thickness of the sc tissue. All of the sites were checked daily by one of the authors. After starting 119 sites in 45 patients, the mean duration of the site was 7.3 ± 5.2 days (range, 1–29). The duration of the site was not affected by age, sex, dose, and type of narcotic or triceps skinfold. The most frequent local toxic effects were redness and swelling. No episodes of serious local toxicity were found. These results suggest that most patients will require a weekly change in the site of infusion. This is a guideline of importance for patients receiving CSCI at home.

In addition to local toxicity at the site of infusion, CSCI will also induce systemic side effects similar to those associated with CIVI. However, three studies have found that the incidence of side effects (mainly sedation, nausea, and confusion) may be lower with CSCI than with intermittent administration (21,26,27). Patients who experience bolus effects (17) are most likely to benefit from CSCI. Respiratory depression is always possible, particularly in patients with compromised renal function and other metabolic abnormalities (38), but it has not been reported as a complication of CSCI by other authors. In 78 consecutive patients we have had only one case (an 85-year-old patient who improved upon discontinuation of narcotics for 2 hours and was restarted at a lower dose; her renal and liver functions were normal). Respiratory depression is less likely to occur in cancer patients who usually, before starting CSCI, have already been exposed to significant doses of narcotics and have, therefore, developed a certain degree of tolerance.

Some authors have suggested that tolerance might develop faster in patients receiving parenteral narcotics, particularly continuous infusions (8,11–13,35,36). We measured the daily increase in the dose of narcotics in 38 consecutive patients who received a CSCI for > 4 days and underwent no other procedure for pain control. The mean daily increase was 2.6% ± 1.4% of the initial dose. Only six patients (16%) had a daily increase $> 5\%$. In these six patients, adequate pain control was

maintained by frequently increasing the dose (28). These results suggest that tolerance is not a clinically relevant problem of CSCI. Moreover, in cancer patients, it may be impossible to differentiate genuine tolerance from an increase in nociceptive stimulation caused by tumor growth.

GUIDELINES FOR CSCI OF NARCOTICS

Patient Selection

Patients who cannot tolerate oral narcotics because of nausea or vomiting are candidates for CSCI, as well as patients who require an extremely large amount of oral narcotics, which is awkward and uncomfortable to administer. Although all of these patients could also be managed with regular injections, it seems clear that CSCI is more effective, simpler, possibly associated with less side effects than intermittent injections, and certainly more convenient for the patients and their families.

Patients who present with the bolus effect are also likely to benefit from CSCI. If a patient is going to be discharged home, CSCI should be preferred to CIVI. However, patients who are already receiving an iv infusion with drugs that are compatible with narcotics can be easily managed with CIVI. Before discharging a patient on CSCI, it is important to ensure that the patient and family have been properly instructed on the method, that there is a care provider at home, and that the patient and family have a phone number for consultation on a 24-hour basis. In part because of the high prevalence of cognitive disorders in terminal cancer patients (37), we have found it very useful to admit our patients for 48 hours at the beginning of the infusion for training and rehearsal of the method with the patient and family.

Those patients whose pain is unlikely to respond to narcotics (neuropathic or episodic pain, patients with total suffering) are poor candidates for CSCI.

Patients with renal failure can present with severe sedation or respiratory depression because of accumulation of morphine metabolites (38). These patients will develop toxicity with any regular form of administration of narcotics, including CSCI. We have recently treated a 41-year-old patient with multiple myeloma with severe bone pain and renal failure (creatinine clearance, 20 ml/min). Because of severe pain with no response to intermittent morphine injections as needed, we started CSCI of hydromorphone (50 mg/day). Within 72 hours of infusion, the patient developed severe sedation and confusion that improved completely after 96 hours of discontinuation of hydromorphone. He was restarted on regular injections of 8 mg iv every 4 hours and in 24 hours he again developed severe sedation and confusion. He developed similar symptoms when changed to morphine regularly. Finally, his pain control was adequate with hydromorphone at 4 mg iv as needed, which he usually received every 18–24 hours. This case suggests that in addition to morphine, hydromorphone can also cause severe toxicity in patients with renal failure. The presence of renal failure represents one of the few indications for the use of as-needed administration of narcotics.

Choice of Drug and Dosage

Most of the reports have used morphine, hydromorphone, or diamorphine. These drugs have a short half-life in common, and therefore steady-state plasma levels (four to five half-lives) will be achieved faster. Although drugs with longer half-lives such as methadone or levorphanol have also been used (24), these drugs have the risk of poor pain control at the beginning of the infusion and delayed toxicity because of the slowly increasing blood level for days after the infusion has started. This is especially important in the case of elderly patients. Therefore, morphine and its congeners should be the first-choice drugs for CSCI. Patients who will be treated with portable pumps will require highly concentrated solutions infused at a very slow rate. In these patients hydromorphone may be preferable because of its higher solubility.

The first step for dose selection is to determine the daily dose of narcotic that the patient is receiving. That daily dose is translated to the equivalent dose in milligrams of parenteral morphine using any of the available tables. If the patient was in adequate pain control, the dose is divided by 24 and the result is administered hourly sc. If the patient was not in adequate pain control, we usually consider an increase of 20%–30% in the daily dose when we start CSCI. An initial bolus dose equivalent to 2 hours of infusion is useful in increasing the blood level of the narcotics, therefore reducing the time interval before reaching steady state. Orders should also be provided allowing as-needed doses of narcotics for breakthrough pain. The usual dose we use is the equivalent of 2 hours of infusion. Both the initial bolus and the extra doses can be administered sc using the same site of infusion via a three-way stopcock. The use of the same site of infusion for extra doses of narcotics does not decrease the duration of the site (34). Once the infusion has been started with morphine or hydromorphone, steady-state blood level will be reached within 24 hours; therefore, if patients do not get adequate pain control or toxicity within 1 day, both events are unlikely to occur later on the same dose of drug. Although in most patients it will be necessary to make daily changes in the dose until adequate pain control is achieved with minimal toxicity, once patients are discharged, our experience in 42 patients suggests that changes are needed every 14 ± 9 days. Less than 20% of the patients will need a rapid increase in the dose of narcotics that can make outpatient management very difficult.

Method of Infusion

Once the drug and dose have been decided, the infusion is started using a 25- or 27-gauge butterfly needle,

usually under the skin of the anterior chest or abdominal wall. As we stated before, the same site can also be used for the administration of the initial bolus and any extra doses of narcotics. The same three-way stopcock can also be used for the administration of steroids or metoclopramide if necessary (39).

We usually start our infusions at a rate of 2 ml/hour. Our data suggest that a volume of up to 10 ml/hour can be administered without pain at the site of infusion. Because of the slow rates of infusion, it is necessary to use a flow-calibrated infusion pump, either portable or nonportable. In small hospitals or in most developing countries, these pumps are not available. Hayes (40) has recently demonstrated that it is possible to administer morphine and hydromorphone safely on a CSCI at rates of 20–80 ml/hour when using hyaluronidase (Wydase) in the infusion (600 units/L of ⅔–⅓ solution). Wydase is an enzyme that hydrolyzes hyaluronic acid, causing rapid diffusion and absorption of injected fluids by temporarily lysing the normal interstitial barrier, which mainly consists of hyaluronic acid. This method was used during the 1940s and 1950s, but after some negative reports on its effects (41,42), it was completely replaced by iv infusions. In recent years it has been rediscovered following research in geriatric groups (43,44). This method not only allows the nurse to control the rate of infusion with a dropper, but also, because of the high rates of infusion, can be used for hydration. When dextrose solution, with or without Wydase, is administered by hypodermoclysis, fluid is drawn from the surrounding tissues into the site of injection. This effect may persist for several hours causing unnecessary pain and a decrease in plasma volume. This danger can be easily avoided by using dextrose solution that contains electrolytes. The cost of Wydase ($2 Canadian per 150-unit vial) and occasional sensitivity reactions are the only drawbacks of this technique.

Selection of Infusors

When the patient will be managed at home, it is important to consider which of the different portable pumps is most suitable for each patient and also for each institution.

Travenol Infusor

This is a disposable plastic cylinder that measures 16 cm in length and 3 cm in diameter and weighs 90 g when full. It infuses a total volume of 48 ml in 1 day at a fixed rate of 2 ml/hour and has a reserve of 12 ml in case of emergencies. Its main advantage is its extreme simplicity and comfortable size (patients keep it attached to their clothes with a pin). Nursing staff and patients can easily be taught to use the infusor. Each day the patient or a relative connects a new infusor to the butterfly needle. We usually provide a 1-week supply at a time. Its main disadvantages are price ($22 Canadian) and the fact that it is useful only for 1 day. Our group will soon start testing a 5-day infusor that has the same size and pumps at a fixed rate of 0.5 ml/hour.

Pharmacia 5200

This is a battery-operated, computer-driven pump that was designed specifically for the purpose of pain control. It not only administers a continuous infusion but also allows the patient to self-administer extra injections with a preset interval and volume. We have tried this pump in four patients and found it safe and reliable. At the present time, we are conducting a comparative trial between this pump and the Travenol Infusor. It can be used with 50- and 100-ml bags. It is heavier and bulkier than the Travenol Infusor and needs to be carried in a holster. Its main drawbacks are the price ($3400 Canadian) and the fact that it is a complicated device that requires more complex teaching for patients and nursing staff. We think that it is necessary to have a specially trained nurse to operate a program with this pump. Therefore, it is unsuitable for small hospitals.

Cormed Pump

This is a battery-operated pump that was originally developed for the administration of chemotherapy. Though simpler and less expensive than the Pharmacia 5200 ($2200 Canadian), it is still complicated and requires a specially trained nurse to be available in case of problems. Also, it does not allow the self-administration of extra injections of narcotics. Coyle et al (24) have recently reported excellent results with this pump.

Autosyringe

This is a battery-operated syringe-driver. Syringe-drivers are mostly used in Britain (20,21–29). Its main advantages are cost ($1900 Canadian) and simple operation as compared to the Cormed or Pharmacia models. However, it is very bulky, with an uncomfortable holster, and not very safe. An ambulatory patient could accidentally self-administer an overdose of narcotics. Even for bedridden patients it may not be very safe during night hours. The change in the syringe is complicated and is performed with difficulty by a patient or relative.

When a CSCI is necessary in an outpatient, it is desirable to start the infusion during a 48-hour admission. The admission will allow for proper dose titration and teaching, as well as for an assessment of the patient's and family's ability to manage the technique. However, we have started six patients on CSCI as outpatients with good results using the Travenol Infusor. When an analgesic procedure has been performed (radiotherapy, chemotherapy, and neurosurgical) and the patient is presumed to be able to manage with oral narcotics, we usually decrease first CSCI to the minimal analgesic dose over 3–4 days and then we change the patient to oral narcotics over 5 days (eg, Day 1—CSCI 80% and

orally 20%; Day 2—CSCI 60% and orally 40%; Day 3—CSCI 40% and orally 60%; Day 4—CSCI 20% and orally 80%; and Day 5—CSCI 0% and orally 100%). Finally, when an admitted patient has good pain control on CSCI, we increase the daily dose by 20% before discharge because of probable increased activity at home.

CONCLUSIONS

More than two-thirds of advanced cancer patients require narcotic analgesics. For patients requiring parenteral narcotics, a continuous infusion of narcotics is safe, has fewer side effects, and is also probably more effective than intermittent injections. The use of the sc route provides equianalgesic results to the iv route with more patient comfort and less potential toxicity. It also allows safe home discharges of patients who otherwise would need prolonged hospitalization. The method of administration of CSCI must be decided according to patient needs and characteristics of the institution where the treatment takes place. More research is needed into CSCI to better define the pharmacokinetics and the clinical effects of drugs administered with this technique, and to compare CSCI to new methods such as patient-controlled analgesia (45). The administration of other drugs such as steroids or antiemetics (39) via the CSCI should also be further explored. We conclude that CSCI is safe and effective and should be regarded as the first choice in patients requiring parenteral analgesia.

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Biochemistry of Azacitidine: A Review[1]

Alice B. Glover and Brian Leyland-Jones[2,*]

Azacitidine is a pyrimidine ring analog of cytidine that is incorporated into RNA causing alteration in RNA synthesis and processing and resulting in inhibition of protein synthesis. Azacitidine as the deoxynucleotide is also incorporated into DNA inhibiting its synthesis and blocking cytosine methylation by noncompetitive inhibition of DNA methyltransferase. The resulting hypomethylation of DNA is thought to induce gene activation and expression and cell differentiation. This may be an underlying factor in azacitidine's antileukemic activity and also contributes to its carcinogenic and tumor-promoting properties in experimental models. [Cancer Treat Rep 71:959–964, 1987]

During the course of preparing a review of the progress of azacitidine over the last 10 years, it was noticeable that whereas its clinical development as an antileukemic agent has in many ways been disappointing (1), there has been considerable continuing interest in the biochemical mechanism of action of azacitidine, particularly its influence on gene expression and cell differentiation. This paper presents an overview of advances in this area with emphasis on clinical implications in the use of azacitidine.

CHEMICAL AND PHARMACEUTICAL CONSIDERATIONS

Azacitidine is an analog of cytidine with a nitrogen atom replacing the carbon at the 5 position of the pyrimidine ring. This substitution has two chemical sequelae which are of potential importance for its biological activity. First, it renders the ring chemically unstable leading to spontaneous decomposition of the compound in neutral or alkaline solutions, with a half-life of approximately 4 hours. Early work suggested that this degradation also occurs after incorporation into nucleic acids and results in instability of the DNA, local uncoiling of specific chromosome segments, and chromatid aberrations (2,3).

Second, the nitrogen atom cannot be methylated and incorporation of azacitidine into DNA results in hypomethylation of the DNA (4). As shown below, this hypomethylation of DNA appears in several different systems to be correlated with gene activation and cell differentiation (5–8).

Clinically, this rapid decomposition of azacitidine in alkaline or neutral solutions means that it must be freshly prepared prior to administration or formulated at a slightly acid pH in Ringer's lactate (pH 6.2) in which it has a half-life of 65 hours at 25°C (9).

CELLULAR PHARMACOLOGY: MECHANISMS OF CYTOTOXIC ACTION AND RESISTANCE

Several steps in cellular metabolism are affected by azacitidine and have been previously reviewed (9–12).

Phosphorylation

Azacitidine is transported into mammalian cells by the same facilitated nucleoside transport system as operates for uridine and cytidine. It is a rapid, saturable process (13). Intracellular azacitidine undergoes three sequential phosphorylation reactions to reach its presumed active form, azacitidine triphosphate.

The first of these steps is catalyzed by the enzyme uridine-cytidine kinase. This is the rate-limiting step in azacitidine metabolism and is subject to potent feedback inhibition by uridine and cytidine triphosphate (UTP and CTP) (13–16). The addition of either cytidine or uridine to cultures of mouse leukemic L1210 cells protected against growth inhibition by azacitidine (14,17). Unfortunately, preliminary studies examining the possible use of cytidine as a rescue agent (18) do not appear to have been pursued.

Since both UTP and CTP are feedback inhibitors of uridine-cytidine kinase, it has been postulated that drugs capable of reducing intracellular UTP and CTP pools would cause enhanced phosphorylation of azaciti-

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dine because of reduced inhibition. This has been shown with two drugs in both murine and human leukemic cell lines. Pyrazofurin, a potent inhibitor of orotidylate decarboxylase, catalyzes the formation of uridine monophosphate from orotidine monophosphate (OMP) in the de novo pyrimidine synthetic pathway (13,19). Deazauridine is an inhibitor of CTP synthetase (20). Earlier work had shown that thymidine and deoxycytidine also enhanced the action of azacitidine in inhibiting growth of murine leukemia cells (17), although the basis for the effect was not clear.

Animal studies to explore these potentially useful interactions have not been pursued so far. One clinical trial of azacitidine in combination with pyrazofurin has been carried out (21), but because this was a phase I study with small numbers of evaluable patients, it is impossible to comment on the clinical efficacy as compared with azacitidine alone in acute nonlymphocytic leukemia.

Resistance to azacitidine has been found to be correlated with a decreased level of uridine kinase activity in the resistant cells both in vivo and in vitro and cross-resistance to other agents requiring uridine-cytidine kinase for activation has been confirmed in vitro (13,22-24).

Finally, azacitidine may be deaminated by the enzyme cytidine deaminase to the derivative 5-azauridine (which may be active) and this process is inhibited by the enzyme hydrouridine (25).

Incorporation Into RNA and Effects Upon Its Processing and Function

For transfer RNA (tRNA), the effects of incorporation of azacitidine on processing and function of RNA are as follows: (*a*) formation of inhibitory, low-molecular-weight (4-7S) RNA (28,37); (*b*) inhibition of tRNA cytosine-5-methyl transferase resulting in synthesis of undermethylated tRNA (34,36,37,42); and (*c*) inhibition of acceptor activity of tRNA (10,42). For ribosomal RNA (rRNA), the effects are as follows: (*a*) inhibition of formation of ribosomal 28S and 18S RNA from 45S precursor RNA (→ inhibition of protein synthesis) (27,29, 30,32,90); (*b*) inhibition of synthesis of nuclear and cytoplasmic rRNA (33); (*c*) inhibition of methylation of nuclear and cytoplasmic rRNA (33,42); and (*d*) instability of polyribosomes → disassembly (10,31,33,35).

After phosphorylation, azacitidine competes with CTP for incorporation into RNA (26).

The cytotoxic effect of azacitidine is widely considered to be due primarily to its incorporation into RNA, with resultant interference in the synthesis of nucleic acids and proteins (10,14,27,28). Studies in Novikoff hepatoma cells showed that azacitidine inhibited the processing of 45S nucleolar RNA to form ribosomal 28S and 18S RNA (29,30), whereas in HeLa cells, Reichman et al (31) found that 45S precursor RNA was completely degraded or altered to an unrecognizable form after treatment with azacitidine. 32S RNA was found to accumulate in human colon carcinoma cells treated with azacitidine, but this effect was unrelated to inhibition of protein synthesis (32).

Azacitidine inhibits the synthesis and methylation of nuclear rRNA (26,33), but does not inhibit the synthesis and methylation of nuclear poly(A)RNA (34). Similar effects of azacitidine on the cytoplasmic species of rRNA have been seen, together with inhibition of tRNA receptor activity and tRNA methylation (34,37); the synthesis of cytoplasmic poly(A)RNA in BSC-1 cells was not inhibited (35). Specifically, azacitidine inhibits activity of tRNA cytosine-5-methyl transferase, resulting in synthesis of tRNA lacking in 5-methylcytidine (36). This inactivation appears to be due to a low-molecular-weight RNA fraction (4–7S) incorporating azacitidine (27,37).

Azacitidine has been demonstrated to inhibit protein synthesis in normal eukaryotic cells (10,38,39) and in cancer cell lines (14,40,41). This has been attributed to the effects on processing and function of RNA shown above as well as the decreased acceptor activity of transfer RNA after incorporation of azacitidine (10,42) and the azacitidine-induced disaggregation of polyribosomes (10,31,35,43).

Phosphorylated azacitidine also blocks de novo synthesis of pyrimidines by inhibiting orotidine-5′-phosphate decarboxylase, resulting in lower uptake of orotic acid for RNA synthesis (10).

Incorporation Into DNA

Effect on DNA Synthesis and Relation to Cytotoxicity

Although azacitidine is incorporated into DNA to a lesser extent than into RNA, its inhibitory effect on DNA synthesis, as measured by incorporation of tritiated thymidine or deoxyadenosine, is greater than its effect on RNA synthesis at the same concentrations in L1210 leukemia cells (14). In rats, when administered shortly after partial hepatectomy, azacitidine led to complete inhibition of DNA synthesis in regenerating liver (39), although if azacitidine was administered before hepatectomy, increased mitotic activity and thymidine incorporation was observed (44).

In L1210 cells the azacitidine-induced inhibition of DNA synthesis, but not of RNA synthesis, paralleled the inhibition of mitosis with lethality predominantly in the S phase (45,46). These findings suggest that inhibition of DNA synthesis does contribute to the cytotoxicity of 5-azacitidine.

Effect on Methylation of DNA and Link to Gene Expression and Cellular Differentiation

After incorporation into DNA, azacitidine inhibits DNA methyltransferase noncompetitively (47–49), causing a block in cytosine methylation in newly replicated DNA (10,50,51) but not in resting, nondividing cells (49). This inhibition of methylation occurs at concentrations which do not cause major suppression of DNA synthesis, as measured by thymidine incorporation (52,53).

There is now a large body of evidence (5–8,54,55) which proposes that alterations in the pattern of cyto-

sine methylation are causally related to gene expression and cell differentiation. Azacitidine, because of its demethylating effect on replicating DNA, has become a useful tool to study the role played by methylation in differentiation and gene activation. An early indication of the ability of azacitidine to induce gene expression in an endogenous virus came from a study by Vesely and Cihak (56). They found that azacitidine given during the first 6 weeks of life markedly hastens (9 weeks as compared with 4–9 months in controls) the appearance of leukemia in AKR mice, a strain that indigenously harbors a leukemia virus. This induction of viral expression in AKR cells by azacitidine has been confirmed in vitro at a level thought not to be causing mutagenic effects (57). More recently, as summarized in table 1, azacitidine has been found to induce cell differentiation and heritable changes in gene activity in several in vitro cell lines, although not in the inactive and X chromosome in human fibroblasts (58) or in the H2-K gene in an embryonal carcinoma cell line (59). In vivo, azacitidine stimulates fetal hemoglobin production in anemic baboons and in patients with severe hemoglobinopathies. This latter effect has been attributed to the ability of azacitidine to produce hypomethylation of DNA in the gamma-globin-gene region, leading to activation of latent gamma-globin genes (60–67). This explanation has, however, been questioned on the basis that hydroxyurea, which is not a hypomethylator, also produces rises in fetal hemoglobins in anemic baboons (68).

Although there are a large number of studies linking demethylation with gene expression, others have found that azacitidine-induced demethylation of DNA may be a necessary but not sufficient condition per se to induce expression at certain loci (48,69–72).

Lu and Randerath (49) used an in vivo model to examine the persistence of azacitidine's hypomethylating effect. They examined the effects of azacitidine on DNA methylation in normal and regenerating mouse liver and L1210 cells grown ip. They found that in repeated administration to intact animals it was extremely difficult to decrease DNA methylation level in liver and in L1210 cells to $< 83\%$ and $< 50\%$, respectively, of control, that only a fraction of the unmethylated sites could be maintained indefinitely in vivo, and that after cessation of prolonged treatment with azacitidine, there was instability in DNA methylation levels in progeny cells.

A finding of possible clinical significance is that pretreatment of L1210 leukemia cells in vitro with azacitidine transiently enhances their susceptibility to DNA single-strand breakage induced by the intercalating agent amsacrine. This effect was attributed to hypomethylation-induced alteration in DNA conformation, making it a better target for intercalation (73). Azacitidine has been widely used in clinical trials of two-drug combinations with amsacrine, but not in trials where the design would permit any conclusions to be drawn as possible synergistic, or even additive, effects (1).

The development of azacitidine as a differentiating agent in leukemia has not been of great interest clinically because of the lack of selectivity of the drug. At the maximal tolerated doses being used clinically it is presumed to be acting via its cytotoxic effect. Levels of ^{14}C-azacitidine in plasma measured during continuous infusion of 50 mg/m^2 were in the range of 0.3–0.5 μg/ml (approximately 1–2 mM) and plasma/leukocyte ratio was around 1 (74). Inhibition of DNA methylation and induction of differentiation have been noted in a mammalian embryonal cell line at 2 μM (4) and in a human

TABLE 1.—Induction of gene expression by azacitidine*

System	Species	Ref Nos.
Plant tumor	Flax plant	91
New mesenchymal phenotypes (muscle, fat, cartilage) including embryonal carcinoma cell lines	Mouse	6,7,50,69,78,79,86,92–98
	Various lines (mouse/human)	99
	Rat (sarcoma)	100
	Chinese hamster	76,77,101
	Human	102–104
Thymidine kinase	Mouse, Chinese hamster	105,106
Blood cell differentiation	Mouse	
	Erythroid	50,107,108
	T-cells	109
	Human	
	K-562	75,110
	T-cells	111,112
Fetal hemoglobin	Human	60,61
Endogenous and exogenous viruses	Review	113
	Mouse	114,115
Alpha-fetoprotein	Rat	116

*This table is essentially an update of Jones, Taylor, and Wilson (ref 6, pg 291, table 1), and references from that review have not been included here.

leukemic cell line at 10 μM (75), whereas concentrations required for cytotoxic effects in L1210 cells in vitro were around 1 mM (33).

While it is not clear how the demethylating and differentiating effect relates to clinical antileukemic activity, there are several studies linking the carcinogenic, tumor-promoting and tumor progression effects of azacitidine in experimental models to the hypomethylation of DNA. In Chinese hamster embryo fibroblasts, a correlation was found between tumorigenicity, trisomy for chromosome 3g, and specific demethylation of HpaII sites on certain genes (76,77).

Studies in both murine and human tumor cell lines have indicated that altered DNA methylation patterns may be linked with the development of tumor heterogeneity and the emergence of subclones with altered sensitivity to differentiating agents and altered tumorigenic and metastatic behavior (78–82).

In the rat, azacitidine has been found to induce various hematological and solid tumors and to act as a tumor promoter but not a complete carcinogen in inducing hepatocellular carcinoma (83,84).

On the other hand, demethylation and differentiation induced by azacitidine have also been linked with reversal of a transformed phenotype to the unprogressed state in adenovirus transformed rat embryo cells (85) and with tumorigenic suppression in murine cell lines (86), indicating that the same mechanism may well contribute to its carcinogenic and antineoplastic properties.

CONCLUSIONS

Over the last decade there has been a considerable increase in knowledge about the biochemical effects of azacitidine. In some areas this has produced promising leads which still need to be pursued preclinically and clinically. There are, for instance, several potentially useful interactions (protective effect of nucleosides and enhancement of cytotoxicity by other drugs and nucleoside analogs), of which only one, pyrazofurin, has been put into clinical trial. Furthermore, the azacitidine derivatives 5,6-dihydro-5-azacitidine (87), 5-aza-2′-dCyd (88), and arabinosyl 5-azacytosine (89) exhibit both differences as well as similarities with the parent compound both with respect to metabolism and biologic effects. Comparisons of these derivatives might shed light on the biochemistry of azacitidine.

The major insights have developed from the discovery that azacitidine causes hypomethylation of DNA. This hypomethylation induces gene activation and expression and cell differentiation. Although the therapeutic significance of its differentiating effect has not been actively investigated (clinical trials have been conducted at maximal or cytotoxic doses), the differentiating effect has been shown to contribute to azacitidine's carcinogenic and tumor-promoting activity in experimental models.

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BRIEF REPORTS

Phase II Trial of Recombinant Beta Interferon in Advanced Colorectal Cancer[1,2]

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Betaseron is a genetically altered recombinant beta interferon with in vitro properties equivalent to those of native beta interferon. Nineteen patients with measurable advanced colorectal carcinoma who had no previous chemotherapy were given 30×10^6 IU Betaseron by iv push on Days 1–5 and 8–12 of each 28-day cycle. One of 17 evaluable patients had a complete response after nine courses of treatment which has been sustained > 9 months. The overall response rate was 6% (95% confidence limits, 0%–18%). Treatment was well tolerated with toxic effects consisting of fever, flu-like symptoms, nausea and vomiting, and transient mild granulocytopenia and liver function abnormalities. All toxic effects were World Health Organization (WHO) grade ≤ 2. No interferon neutralizing antibody activity was detected. Phase I and preclinical data support further investigation of Betaseron with dose escalation to tolerance for individual patients, as well as investigation of concomitant therapy with 5-fluorouracil, in an attempt to improve the observed response rate in colorectal cancer. [Cancer Treat Rep 71:965–967, 1987]

Interferons represent a family of protein and glycoprotein compounds produced by most nucleated cells in response to both viral and some types of nonviral challenge. With recombinant technology, a variety of interferons, classified antigenically as alpha, beta, or gamma, are now available for use in the clinic. Native beta interferon derived from fibroblasts exhibits characteristics similar to alpha interferon (1,2). Previous trials with alpha interferons in colorectal cancer have reported response rates of 0%–10% (2). We report a phase II study of recombinant beta interferon in metastatic colon carcinoma.

MATERIALS AND METHODS

Recombinant human beta interferon (Betaseron) provided by Triton Biosciences (Alameda, CA) has a specific activity of 2×10^8 IU/mg protein. Produced by *Escherichia coli*, serine has been substituted for the cysteine residue at position 17 of the native molecule resulting in in vitro properties similar to native human beta interferon (3).

Between June 1985 and June 1986, 19 patients with pathologically documented metastatic adenocarcinoma of the colon were entered on study. None of these patients had received prior chemotherapy or therapy with biologic response modifiers. Resection of their primary tumor was the initial therapy in all patients. Prior to entry, all patients had a baseline physical examination, cbc, urinalysis, chemistry profile, chest x-ray, electrocardiogram, and tumor measurement by liver-spleen and/or computerized tomographic (CT) scan. Eligibility criteria included acceptable hematopoietic function (wbc count > 4000/mm^3, granulocyte count > 2000/mm^3, hemoglobin > 9 gm/dl, hematocrit > 25%, and platelet count > 100,000/mm^3). Additional requirements included acceptable renal function (BUN ≤ 25 mg/dl; creatinine < 1.5 mg/dl) and acceptable liver function (total bilirubin < 1.5 mg/dl; SGOT and alkaline phosphatase < 2 times upper limits of normal) unless liver

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metastases were present. Concomitant administration of aspirin, nonsteroidal anti-inflammatory agents, steroids, or other chemotherapeutic agents was not permitted.

Therapy was administered in 4-week cycles. An iv bolus injection of 30×10^6 IU Betaseron was given on Days 1–5 and 8–12 with a subsequent 2-week rest period. Acetaminophen, 650 mg, was given prior to dosing and as needed, every 3 hours, for relief of fever and flu-like symptoms. Toxicity was graded according to World Health Organization (WHO) criteria.

Tumor measurements were done monthly and patients who showed disease progression were removed from study. Responses were classified by the standard definitions (4). Interferon neutralizing antibody activity was assayed by ELISA on a monthly basis, and, when possible, 1 and 3 months after treatment termination. The IgG and IgM titers were expressed both as OD415 × serum dilution and as micrograms of immunoglobulin per ml of patient serum. The detection limit of the assay is 3 μgms/ml; the detection limit for IgM is 8 μgms/ml. Of 18 patients tested, none were positive for IgM. Three tested positive (> 15 μgms/ml) for IgG and three had barely detectable values of IgG (4–15 μgms/ml). Neutralization titers were determined for samples with positive immunoglobulin titers with results expressed as number of units of Betaseron neutralized by 1 ml of patient serum (IFN) in an antiviral assay. The detection limit of the assay is 13 NU/ml. All values were the mean of three determinations.

RESULTS

As listed in table 1, all 19 patients entered were evaluable for toxicity; 17 were evaluable for response. One patient who required surgery for a small-bowel obstruction secondary to adhesions was removed from study after one cycle of treatment. Another patient refused further treatment after one cycle although there was no toxicity. Disease progressed while on therapy in 16 of 17 patients evaluable for response. Progression occurred after one cycle of therapy in four patients, after two cycles in nine patients, and after three cycles in three patients. One patient achieved complete response after 9 months of therapy, with complete regression of liver metastasis as measured by CT scan. This patient remains on study with a complete response sustained > 9 months. It is of note that this response involved the resolution of 10 measurable lesions within the liver, with the initial sum of the products of the perpendicular diameters measuring 11 cm^2.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Sex	
Males	10
Females	9
Median age in yrs (range): 63 (20–85)	
ECOG performance status	
0	11
1	8
Prior treatment	
Surgery	19
Radiation therapy	4
Chemotherapy	0
Metastatic site	
Liver	17
Lung	1
Presacral mass	1
Other intra-abdominal	2

Fever and flu-like symptoms occurred in all patients, but were WHO grade ≤ 2. Tachyphylaxis to these symptoms was noted within each 10-day treatment cycle but generally did not occur from one cycle to the next. Of the nineteen patients enrolled, eight had nausea and vomiting (WHO grade ≤ 2) at varying times during treatment, and four experienced leukopenia (WHO grade ≤ 2) during the second and subsequent cycles of therapy. Four patients experienced mild hepatotoxicity (WHO grade ≤ 2), as manifested by reversible asymptomatic elevation of SGOT, during the second and later cycles. Mild transient proteinuria was noted in two patients. One patient had prior chronic hypertension and mild pretreatment elevation of creatinine. In a 24-hour urine collection taken after three cycles of therapy total urine protein was 1.4 gm. None of the 19 patients enrolled experienced grade 3 or 4 toxicity. None required dosage reduction, and no one was removed from study due to toxic effects. No weight loss > 10% of baseline was noted. No patients had measurable interferon neutralizing titers.

DISCUSSION

The response rate in metastatic colon cancer for Betaseron therapy in this study was 6% (95% confidence limits, 0%–18%). Treatment was well tolerated and safety experience was consistent with that previously reported for interferon preparations (5). In previous clinical trials with both human and recombinant alpha interferons in the treatment of advanced colorectal carcinoma, response rates varying from 0% to 10% have been reported (2). It is clear that biologic activity can differ between interferon preparations, and that it might be schedule dependent (6). Though the response rate on this study suggests minimal antitumor activity, several significant conclusions can be made. A sustained complete response suggests that Betaseron is active in colorectal cancer. This response was obtained over a longer period of time (9 months) than is usually seen with traditional chemotherapeutic agents, suggesting that trial duration may be critical in evaluating biologic response modifiers. Though no significant chronic toxicity was observed, there was interpatient variability in toxicity to Betaseron.

Future trials that might define patient characteristics that correlate with Betaseron toxicity and antitumor

activity are warranted. Phase I and preclinical data suggest approaches that might increase the minimal antitumor activity observed for Betaseron in this trial (7). Because the dose-response curve for Betaseron has not yet been defined, studies that allow for dose escalation to tolerance in individual patients should be done. In addition, in vitro data suggesting antitumor synergy between interferons and 5-fluorouracil warrant phase I–II trials evaluating concomitant administration of Betaseron and 5-fluorouracil (8).

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Phase II Evaluation of Oral Medroxyprogesterone Acetate in Advanced Breast Cancer: A Northern California Oncology Group Study[1,2]

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Medroxyprogesterone acetate (MPA) is a semisynthetic progestin possessing high progestational activity and has been used in the treatment of advanced breast cancer (1). Early studies with low doses (100–266 mg/day) of oral MPA demonstrated response rates of 0%–25% (1–3). More recent studies using high-dose (500–1500 mg/day) MPA given im produced response rates of approximately 40% (4,5). Unfortunately, high doses of injectable MPA may be disadvantageous for long-term use. The present study reports the results of using oral high-dose MPA in postmenopausal women with previously treated metastatic breast cancer.

MATERIALS AND METHODS

From February 1982 to November 1984, 35 patients were entered in this study of the Northern California Oncology Group. Patients were required to be at least 1-year postmenopausal and to have pathologically documented breast cancer and measurable metastatic disease refractory to at least one chemotherapy regimen or that became refractory after response to at least one hormonal manipulation. The patients had to have been off all therapy at least 4 weeks and to have a Karnofsky performance status $\geq$ 60%. Patients were excluded if they had estrogen receptor-negative tumors, other concurrent or past malignancies (except adequately treated basal or squamous cell carcinoma of the skin and in situ carcinoma of the cervix), central nervous system or hepatic metastases, prior unsuccessful hormonal therapy, or therapy with other progestational agents.

MPA (provided by the Upjohn Co, Kalamazoo, MI) was administered orally as 50-mg tablets at a dose of 400 mg/day. Responding patients were continued until disease progression; patients with stable disease continued MPA at the discretion of the treating physician.

Only measurable lesions were used in the evaluation of response to therapy. Complete response (CR) was defined as the disappearance of all known disease for at least 4 weeks; partial response (PR) was defined as a 50% decrease in the sum of the products of the two greatest perpendicular axes of all lesions for at least 4 weeks. PR in osseous sites required recalcification of lytic lesions. Disease progression was defined as enlargement of known sites of disease, appearance of new sites of disease, or appearance of ascites or pleural effusion.

RESULTS

Patient characteristics are shown in table 1. Of the 35 patients entered, three are not evaluable for response by virtue of lack of measurable disease but are evaluable for toxicity and survival. In the 32 evaluable patients, there were three CRs (9%) and six PRs (19%), for an overall response rate of 28%. Responses were seen only in patients with skin and soft tissue lesions. There was a somewhat higher response rate in patients with estrogen receptor-positive tumors.

The median time to response was 8 weeks, with a mean of 9.1 weeks. The median time to disease progression for all patients was 2.8 months and for responding patients was 8.4 months (range, 2.5–13.8). The median survival time was 10.4 months (range, 0.7–38.1).

No significant hematologic toxicity was noted. Five patients were removed from the study because of severe adverse reactions: two of these may have been "tumor

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TABLE 1.—Characteristics and treatment results of evaluable patients

Characteristic	No. of patients (%)
Evaluable	32
Mean age in yrs (range): 63.1 (39–75)	
Karnofsky performance status	
60-70	10 (31)
80-100	22 (69)
Estrogen receptor status	
Positive (≥ 10 fmols/mg of cytosol protein)	19 (59)
Equivocal	1 (3)
Unknown	12 (38)
Dominant disease sites	
Skin/soft tissue	22 (69)
Bone	8 (25)
Visceral	2 (6)
No. of organ sites involved	
1	18 (56)
2	10 (31)
3	4 (13)
Response (CR + PR)	9 (28)
Response by dominant disease sites	
Skin/soft tissue	9 (41)
Bone or visceral	0
Response by estrogen receptor status	
Positive ($N = 19$)	6 (32)
Unknown ($N = 13$)	3 (23)
Response by prior chemotherapy	
Prior chemotherapy ($N = 21$)	6 (29)
No prior chemotherapy ($N = 6$)	1 (17)
Unknown ($N = 5$)	2 (40)
Response by prior hormonal therapy	
Prior hormonal therapy ($N = 23$)	6 (26)
No prior hormonal therapy ($N = 4$)	2 (50)
Unknown ($N = 5$)	1 (20)

flares" when they experienced increased pain, and one each secondary to pulmonary edema, pulmonary embolus, and hypercalcemia. Weight gain > 10% of baseline was observed in six patients and edema of the extremities was noted in three patients. Other side effects have occurred infrequently as shown in table 2.

TABLE 2.—Side effects*

Side effect	No. of patients (%)
Total nonhematologic toxic effects	19 (54)
Weight gain	6 (17)
Edema	3 (9)
Anxiety and nervousness	2 (6)
Vaginal spotting	2 (6)
Headache	2 (6)
Thrombosis/pulmonary embolism	2 (6)
Hypercalcemia	1 (3)
Dysuria	1 (3)
Leg Cramps	1 (3)
Weakness	1 (3)
Vomiting	1 (3)

*$N = 35$ patients.

CONCLUSION

Oral high-dose MPA induced an objective response rate of 28% in the breast cancer patients treated in this study. This result confirms the efficacy of oral high-dose MPA in advanced breast cancer in postmenopausal women (6,7). The relatively low objective response rates may be related to the extent of previous treatment. All objective responses were observed in patients with only skin and/or soft tissue lesions. The lack of response in dominant osseous sites may be related in part to the rigorous response criteria. Significant weight gain was observed, and patients receiving MPA may need diet counseling. Oral MPA may provide a convenient and useful therapeutic option for selected postmenopausal women with advanced breast cancer.

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BRIEF REPORTS

Continuous Venous Infusion of Doxorubicin in Advanced Sarcomas[1,2]

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Seventeen patients with advanced sarcoma were treated with continuous venous infusion of doxorubicin for a mean of 118 days, achieving total doses up to 1097 mg/m^2. Three partial responses and one minor response were obtained. Major toxic effects were stomatitis and hand-foot syndrome. There was a low incidence of leukopenia (18%) and clinical cardiotoxicity (11%). Continuous venous infusion is a safe means of administering doxorubicin, with a response rate similar to that observed with bolus doxorubicin in metastatic sarcoma.
[Cancer Treat Rep 71:971-972, 1987]

Doxorubicin is the most active single agent in the treatment of sarcoma (1), but it is cytotoxic only when cells are actively cycling. Therefore, with a short plasma half-life (200 minutes), bolus doxorubicin is likely to affect only a fraction of malignant cells. Continuous venous infusion (CVI) of doxorubicin may overcome this drawback. Preliminary data from long-term CVI studies suggest that cumulative drug levels > 450 mg/m^2 may be used without cardiotoxicity (2). Our phase I study of CVI doxorubicin showed responses in several patients with sarcoma (3). For these reasons we undertook a phase II study of CVI doxorubicin in sarcoma.

PATIENTS AND METHODS

Seventeen adult patients with metastatic sarcoma, both of soft tissue and of bone, were entered in the study between May 1983 and April 1986, including three patients already reported (3). Patient characteristics are shown in table 1.

Medtronic 8610 programmable pumps (3) were implanted sc in all patients, and the doxorubicin infusion was initiated at 3 mg/m^2/day. The pumps were emptied of residual drug and refilled every 2-3 weeks, due to the instability of doxorubicin in the pump beyond 3 weeks. Doses were escalated until the maximal tolerated infusion rate was reached. Patients were observed clinically and hematologically for toxicity, and cardiac toxicity was assessed clinically and echocardiographically. CVI was continued until there was evidence of disease progression, until discontinuation was mandated by toxicity, or until death. Standard definitions of response were used.

RESULTS

All patients were evaluable for response and toxicity. Doxorubicin was administered by CVI for a mean of 118 days of actual drug infusion (range, 28-212 days). Total dose ranged from 105 to 1097 mg/m^2 (median, 268). Mean dose rate achieved was 3.7 mg/m^2/day (range, 3-5.5). Three patients (two of whom had received previous iv bolus doxorubicin) achieved partial response lasting 4, 11, and 15 months. These three patients had cumulative doses of CVI doxorubicin of 547, 679, and 1097 mg/m^2, respectively, with total cumulative doses of 547, 1142, and 1660 mg/m^2 (including prior bolus doxorubicin). One patient had minor response and four had stable disease, for a median duration of 5 months. The remaining nine patients had disease progression while receiving CVI doxorubicin.

The major dose-limiting toxic effects were stomatitis (82% of patients) and the "hand-foot" syndrome (53%). The latter consisted of painful digital dysesthesia, accompanied by an erythematous, desquamating dermatitis of the hands and feet, which showed a trend towards an increase with the duration of CVI, in terms of both occurrence and severity, but this was not statistically significant. Alopecia was almost invariable (94%). Clinical cardiotoxicity developed in two patients (11%) (total

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TABLE 1.—Patient characteristics

Characteristic	No. of patients
Median age in yrs (range): 50 (25–78)	
Male/female	3/14
Soft tissue/bone sarcoma	13/4
Prior therapy	
Surgery	14
Radiation therapy	9
Doxorubicin-based chemotherapy	6
Non-doxorubicin-based chemotherapy	0
Median interval from diagnosis to CVI therapy in mos (range): 20 (3–132)	
Median survival from start of CVI doxorubicin in mos (range): 8 (2–29+)	

doxorubicin doses, 1660 and 723 mg/m^2). However, subclinical evidence of myocardial dysfunction was seen in two other patients who had slightly reduced ejection fractions by echocardiography (59%–53% and 56%–51%). A third patient had a minor myocardial infarction while receiving therapy. Two further patients had no change in ejection fractions, but had a change in the left ventricular end-systolic wall stress/% fractional shortening relationship (4). Only one patient with echocardiographic dysfunction had a cumulative dose < 300 mg/m^2, and the median dose in patients with echocardiographic dysfunction was 420 mg/m^2. Leukopenia was not a major toxic effect; no patient developed leukopenic sepsis, and only three patients had a wbc count < 2000/mm^3 during therapy (one had concurrent myelodysplastic syndrome, with a wbc count of 2000/mm^3 at entry). Nausea and vomiting were not significant problems. There were no treatment-related deaths.

DISCUSSION

CVI doxorubicin offers several potential advantages over standard intermittent iv bolus; it may overcome relative resistance due to cell kinetic factors, and host toxicity may be advantageously altered. In this study, CVI doxorubicin was an effective regimen in adults with refractory metastatic sarcoma, with a response rate of 22%, comparable to the 18%–34% rates reported for bolus doxorubicin (1). Two of the responders had already received maximal bolus doxorubicin (one eventually developed clinical cardiotoxicity after a total of 1660 mg/m^2). Nausea, myelosuppression, and cardiotoxicity, commonly seen with bolus doxorubicin, were not major problems with CVI. The two patients with clinical cardiotoxicity both had cumulative doses > 550 mg/m^2. Four other patients had evidence of cardiac dysfunction detected only by subtle echocardiographic criteria.

The major toxic effects encountered appear to be specific to CVI administration of doxorubicin. These were stomatitis and the desquamative hand-foot syndrome which appears to be peculiar to long-term continuous infusion of cytotoxic drugs. Hand-foot syndrome was particularly debilitating in patients who had amputated limbs, in whom pain and dysfunction in the remaining limbs was a severe handicap. The syndrome also occurs with CVI 5-fluorouracil (5). However, we have not observed it in 23 patients treated for > 30 days with CVI vinblastine (6).

We conclude that CVI doxorubicin is an effective regimen for metastatic sarcoma, and it may be safely and effectively administered to patients who have already been treated with bolus doxorubicin and have either encountered dose-limiting toxicity or reached a maximal cumulative bolus dose or have relapsed after completing adjuvant therapy with bolus doxorubicin. The regimen is less myelosuppressive, and possibly less cardiotoxic, than conventional bolus administration, although further data are required to define the incidence and severity of cardiotoxicity in comparison with bolus therapy. Side effects specific to CVI therapy may be expected during its use.

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BRIEF REPORTS

Phase I Trial of Procarbazine as a 5-Day Continuous Infusion in Children With Central Nervous System Tumors[1]

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Seven children with previously treated brain tumors were enrolled in a phase I trial of 5-day continuous-infusion procarbazine at 360, 480, and 638 mg/m^2/day. Vitamin B_6 levels were monitored. Myelosuppression was moderate though occasionally delayed, and nausea and vomiting were mild. At the highest dose level, a patient experienced severe psychosis that persisted for several weeks. From that dose-limiting toxicity and the degree of myelosuppression, the recommended dose for phase II trials in children is the same as for adults, 450 mg/m^2/day. [Cancer Treat Rep 71:973–974, 1987]

Procarbazine has shown antineoplastic activity when tested in a variety of tumors as a single agent and in combination with other drugs. Procarbazine has been widely used in combination with mechlorethamine, vincristine sulfate, and prednisone (MOPP) to treat children with Hodgkin's disease (1). The MOPP regimen has also been effective in salvage therapy of children with brain tumors (2) as well as in primary therapy for infants with brain tumors (3). A Pediatric Oncology Group study comparing MOPP with the same regimen without mechlorethamine showed that mechlorethamine does contribute to the remission rate, even though the overall survival rate was not different (4). This discrepancy was largely due to higher toxicity in the mustard-containing regimen. The significant effect of vincristine, procarbazine, and prednisone together underscores the point that vincristine and procarbazine are active against brain tumors. The oral dose of procarbazine has been 100 mg/m^2/day × 10 in MOPP therapy, and its toxicity at this dose level has been tolerable.

The recent availability of procarbazine in an iv form made this drug a natural candidate for evaluation. However, the maximum tolerated dose of iv-administered procarbazine had not yet been determined by a phase I study in children. This study reports on one such effort. An adult phase I study recommended a starting dose of 450 mg/m^2/day × 5 days (5). Reported central nervous system (CNS) toxicity has been said to be related to vitamin B_6 depletion.

PATIENTS AND METHODS

Procarbazine was administered under a protocol approved by our Institutional Review Board. Written informed consent was obtained. All children with recurrent brain tumors who were not candidates for known effective regimens were eligible. Computed tomographic (CT) scan diagnosis was acceptable for inoperable lesions. Patients had to be well nourished and life expectancy had to be sufficient for at least one complete course with 3 weeks of observation.

Procarbazine was given iv by continuous infusion for 5 days, each 12-hour period in up to 250 ml of appropriate iv solutions, protected from light. The starting dose for these children was based on 80% of the adult starting dose. The first three patients received 360 mg/m^2/day. This dose was escalated for the next three patients to 480 mg/m^2/day. The highest dose tested was 638 mg/m^2/day. Escalation in the same patient was allowed after two doses at the level of enrollment. Doses were to be postponed if the patient had an absolute granulocyte count $< 2000/mm^3$ or a platelet count $< 100{,}000/mm^3$ when the next dose was due. Patients were monitored with a cbc plus differential count, liver and renal function tests, coagulation profile, serum electrolytes, urinalysis, and serum vitamin B_6 levels. All patients received a tyramine-free diet. Pediatric Oncology Group toxicity scales were used to rate toxicity (6). Tumor response was gauged by appropriate radiologi-

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TABLE 1.—Patient characteristics

Patient No.	Age*	Sex	Diagnosis	Prior therapy†	Response‡
1	8 yrs, 3 mos	M	Brainstem glioma	Surgery, radiotherapy, carmustine, MOPP	PD
2	11 yrs	M	Choriocarcinoma	Surgery, radiotherapy, CVB, CISCA/VB, VAC, HMTX	PD
3	8 yrs, 4 mos	F	Brainstem glioma	Biopsy, radiotherapy	PD
4	9 yrs, 4 mos	F	Anaplastic astrocytoma	Surgery, radiotherapy, MOPP	PD
5	10 yrs, 2 mos	F	Ependymoma	Surgery, radiotherapy	PD
6	11 mos	M	Medulloblastoma	Surgery × 2, MOPP, radiotherapy, CAPT	PD
7	6 yrs, 6 mos	F	Brainstem glioma	Radiotherapy, CAPT	SD

*Age is at time of registration.

†MOPP = mechlorethamine, vincristine, prednisone, and oral procarbazine; CVB = cisplatin and vinblastine sulfate hydrate; CISCA/VB = cisplatin, cyclophosphamide, doxorubicin, vinblastine sulfate hydrate; VAC = vincristine, dactinomycin, cyclophosphamide; HMTX = high-dose methotrexate; CAPT = carboplatin.

‡SD = stable disease; PD = progressive disease.

cal measures, usually CT with and without contrast and, in selected instances, by magnetic resonance imaging. No concurrent tumor-directed therapy was given except dexamethasone when clinically indicated.

RESULTS AND DISCUSSION

Seven patients were enrolled, two of whom advanced in dose. A total of five courses were given at 360 mg/m^2, five at 480 mg/m^2, and two at 638 mg/m^2. Patient characteristics are shown in table 1. One patient (No. 7) showed radiographically stable disease and neurological improvement after therapy. The remainder had progressive disease.

Nausea and vomiting was mild, never exceeded grade 2, and abated over a 5-day course. Myelosuppression was moderate, though it was delayed until Day 41 in one patient. All others recovered their counts by the time the next course was due. Grade III granulocytopenia occurred in one of five courses at 360 mg/m^2 and two of five courses at 480 mg/m^2. Grade III thrombocytopenia occurred in one of five courses at each of those dose levels. No life-threatening myelosuppression was seen and no infections occurred during times of granulocytopenia.

One patient, 24 hours after completion of the infusion at the highest dose, experienced an acute psychosis manifested by hallucinations, self-mutilation, and panic attacks, and required hospitalization. The serum vitamin B_6 level was normal, and vitamin B_6 administration had no effect. She was intolerant of very low doses of Thorazine and continued to have psychological sequelae. This constituted a severe, dose-limiting toxicity. At that same dose level, thrombocytopenia (< 100,000 cells/mm^3), which resolved, was seen in Patient 6. This patient was an infant who was restless for 2 weeks following completion of the infusion. In a parallel study of adults with gliomas, two of 14 patients had reversible confusion and excitation during procarbazine infusion (W. K. A. Yung, personal communication).

We conclude that the maximum tolerated dose for iv procarbazine administered in this fashion is 480 mg/m^2/day. Since that is close to the adult dose, we would recommend use of 450 mg/m^2/day if further studies are undertaken. The occasional delayed myelosuppression would probably make a schedule for children of every 4 weeks more prudent than the recommended frequency for adults of every 3 weeks.

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BRIEF REPORTS

Continuous-Infusion Cisplatin and Bolus 5-Fluorouracil in Colorectal Carcinoma[1,2]

Marshall R. Posner,* James F. Belliveau, Alan B. Weitberg, Kert Sabbath, Michael C. Wiemann, Francis J. Cummings, and Paul Calabresi[3]

Twenty-one evaluable patients with metastatic colorectal carcinoma were treated with a combination of continuous-infusion cisplatin (25 mg/m^2/day × 3 days) and bolus 5-fluorouracil (400 mg/m^2/day × 3 days). Toxicity was minimal. Seven patients (33%) responded. All responses were observed among the 16 previously untreated patients (44%) and lasted a median of 30 weeks. The results indicate the need for phase III trials of this treatment. [Cancer Treat Rep 71:975-977, 1987]

The chemotherapy of advanced colorectal carcinoma with single and multiple agents has generally yielded less than satisfactory results, with little, if any, improvement over the 15%-25% response rate seen with single-agent 5-fluorouracil (5-FU) (1). Treatment with cisplatin (CP) has resulted in equally poor response rates (2). There is, however, a scientific basis for clinical testing of the combination of these two drugs. It has previously been demonstrated in animal tumor models that treatment with a combination of CP and 5-FU results in a synergistic antitumor effect (3,4). Synergism has been observed in the treatment of head and neck cancer (5). Continuous-infusion CP has been shown to increase the filterable platinum exposure in patients, which may result in an increase in the activity of CP (6). Furthermore, because the mechanism of the synergistic interaction of CP with 5-FU is unknown, there is no compelling evidence to suggest an optimal method of coadministration of these drugs for the treatment of colorectal tumors. Animal models have suggested that the simultaneous presence of both agents gives an improved response (4). On the basis of these observations, we initiated a phase I-II trial of the combination of continuous-infusion CP plus bolus 5-FU for advanced colorectal carcinoma.

MATERIALS AND METHODS

Patients were eligible for this study if they had measurable metastases from histologically documented colorectal carcinoma and recovered from previous therapy. Patients were required to have a creatinine clearance of > 40 ml/minute. Treatment was given with CP as previously described (7). In brief, after prehydration with 0.5 L of 0.9% NaCl over 2 hours, patients were given CP, 25 mg/m^2, daily for 3 days as a continuous infusion with each day's dose divided into 3.0 L of 0.9% NaCl. 5-FU was given as a bolus of 400 mg/m^2 for 24, 48, and 72 hours after the initiation of CP therapy. Posthydration consisted of 0.5 L of 0.9% NaCl with 20 mEq of KCl over 2 hours followed by 0.5 L of 5% dextrose in water with 4 g of $MgSO_4$ over 2 hours. Treatment was given every 5 weeks. Responses were assessed by computerized tomographic (CT) scan and chest radiography using standard criteria (7). Responses and toxicity were reviewed at each cycle.

RESULTS

The characteristics of the patients and the results of the study are summarized in table 1. Between March 1985 and December 1986, 22 patients were entered; 21 were evaluable for toxicity and response. One patient refused therapy after one cycle and was lost to follow-up. There were 13 previously untreated patients, eight patients who had received previous radiotherapy, and five who had received previous chemotherapy, all with 5-FU alone or in combination with other agents or radiotherapy. For the purposes of analysis, those 16 patients who had received no previous chemotherapy were considered

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TABLE 1.—Patient characteristics, results, and toxic effects

	No. of patients
Patients entered	22
Evaluable	21
Mean age in yrs (range): 61 (41–78)	
Previous chemotherapy	5
Previous radiotherapy	8
Treatment cycles	74
Responses	
Complete response	1
Partial response	6
Stable disease	2
Progressive disease	12
Sites of disease (responses)	
Liver	13 (6)
Lung	5 (3)
Abdominal cavity	8 (0)
Carcinoembryonic antigen	11 (6)
Toxicity	
Leukopenia	
< 2000 cells/μl	3
< 1000 cells/μl	1
Thrombocytopenia	
$< 100{,}000$ cells/μl	2
$< 50{,}000$ cells/μl	1
Nausea and vomiting	
Grade 2*	13
Grade 3	1
Neurotoxicity	
Grade 1	3
Nephrotoxicity	
Creatinine > 2.0 mg/dl	1
Diarrhea	
Grade 2	2
Mucositis	1

*All graded toxicity is according to Eastern Cooperative Oncology Group (ECOG) criteria.

previously untreated. The median performance status of the patients was grade 1 as measured by the Eastern Cooperative Oncology Group (ECOG) criteria. Patients were treated with a mean of 3.4 courses (range, one to nine). Severe leukopenia and thrombocytopenia occurred in one patient who had received previous radiotherapy to the pelvis. No infections or bleeding episodes occurred in any patients. One patient experienced reversible renal toxicity as determined by an increase in serum creatinine to 2.1 mg/dl. Nausea and vomiting were easily controlled with a combination of dexamethasone, droperidol, and metoclopramide. Average weight alteration during the chemotherapy was 0.6%. Mild neurotoxicity (paresthesias) occurred in three patients who had received CP doses of > 450 mg/m^2. One patient with diabetes, hypertension, and previous myocardial infarction died of diabetic ketoacidosis and myocardial infarction during therapy. Anal mucositis occurred in one patient with previous radiotherapy to the anal area.

There were one complete response and six partial responses (response rate = 33%; 95% confidence interval, 13%–53%). All responses were seen in the 16 previously untreated patients (response rate = 44%; 95% confidence interval, 19%–69%). Two patients, one previously treated and one previously untreated, had stable disease. Median duration of response was 30 weeks (range, 20–55). The total median survival was 24 weeks (range, 4–96+) with 44 weeks (range, 16–88+) and 20 weeks (range, 3–96+) for responders and nonresponders, respectively. The single complete responder had resolution of liver metastases and normalization of carcinoembryonic antigen.

DISCUSSION

In the present study, we report a response rate of 33% in all patients treated, and 44% in previously untreated patients who received continuous-infusion CP and bolus 5-FU for metastatic colorectal carcinoma. Toxicity was minimal and acceptable although treatment required hospitalization for a total of 4 days. These data indicate that this regimen is at least equivalent to single-agent 5-FU. Others have reported similar response rates to differing schedules of this combination, usually with CP given as a bolus, and 5-FU as a bolus or continuous infusion (8,9).

In vitro studies of CP have indicated that prolonged treatment with low concentrations of drug might be as effective as short treatments with doses an order of magnitude higher (10). Continuous administration of the CP results in a twofold increase in the area under the curve of filterable plasma platinum compared to bolus administration of similar cumulative doses (6). Since the activity of CP may be related to filterable platinum in plasma, continuous infusion may improve the therapeutic ratio of the drug and the antitumor effect. Phase III trials of this or similar regimens will be needed to determine whether there is a significant benefit of this treatment over 5-FU alone and are indicated by the results of this trial.

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BRIEF REPORTS

Efficacy of Continuous High-Dose Metoclopramide in Patients Receiving Daily Cisplatin Infusions[1]

Ghaleb A. Saab,* Nuhad Ibrahim, and Nuhad Azouri[2]

Continuous high-dose metoclopramide (MCP), 2 mg/kg/24 hours, was administered for a period of 84–90 hours to 30 consecutive patients with various advanced solid malignancies receiving concomitantly a 3-day infusion of cisplatin. The latter was given at a dose of 40 mg/m^2/day over 6 hours every day, in combination with doxorubicin and cyclophosphamide. Diphenhydramine and dexamethasone were also administered. This prolonged antiemetic therapy proved to be safe with only a 16% incidence (five of 30 patients) of extrapyramidal side effects not necessitating discontinuation of MCP. Also, antiemetic efficacy was demonstrated with 63% of the patients not experiencing any emesis. [Cancer Treat Rep 71:979–980, 1987]

Cisplatin (*cis*-diamminedichloroplatinum) is one of the most potent emetogenic drugs known, when given by bolus or continuous infusion. Recently, a 3–5-day infusion form has been advocated in order to avoid nephrotoxicity and allow administration of substantially higher doses during each cycle. Metoclopramide (MCP), on the other hand, is the most commonly used antiemetic, singly or in combination, for control and prevention of chemotherapy-induced nausea and vomiting. It has been used as intermittent bolus infusion. No studies have been reported as to the efficacy and optimal way of administering MCP during extended periods of infusional chemotherapy. We report the antiemetic role of MCP when given as a continuous infusion, in combination with diphenhydramine (DPH) and dexamethasone (DXM), in patients receiving multidrug chemotherapy with cisplatin infusions.

PATIENTS AND METHODS

Thirty consecutive patients with a variety of solid malignancies were entered in the study: nine ovarian carcinoma, seven carcinoma of lung, six carcinoma of the prostate, six carcinoma of the urinary bladder, and two malignant thymoma. No patient had received prior chemotherapy. A total of 82 cycles of cisplatin were administered, each at a dose of 40 mg/m^2 iv over 6 hours, repeated once daily for 3 consecutive days. On the third day of treatment, cisplatin was combined with doxorubicin, 40 mg/m^2 iv push, and cyclophosphamide, 650 mg/m^2 iv push. Each cycle was repeated 3 weeks later, blood counts permitting. The number of cycles per patient varied because the study was stopped when patient No. 30 was entered (table 1).

Antiemetic therapy was started 12 hours prior to chemotherapy as follows: MCP, 2 mg/kg/24 hours in 5% dextrose in water by continuous infusion until

TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Entered	30
Evaluable for toxicity	30
Male:female ratio: 1.2	
Median age in yrs (range): 53 (37–70)	
Median Karnofsky performance status (range): 80% (60%–100%)	
Total No. of courses: 82	
Median No. of courses/patient (range): 3 (1–6)	
Courses of treatment	
1	1 (3.3)
2	6 (20.0)
3	15 (50.0)
4	5 (16.6)
5	2 (6.6)
6	1 (3.3)

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12–18 hours following the end of all treatment; DPH, 25 mg by iv push every 8 hours; and DXM, 4 mg by iv push every 8 hours until 12–18 hours following the end of chemotherapy as well. Approval by the Ethical Committee of our University Hospital and oral informed consent from each patient were secured.

Efficacy of antiemesis was evaluated via a standardized patient questionnaire, twice-daily interviews made independently by each author, and written observations made by the nursing staff. Nausea and vomiting were graded (0, no emesis; +1, three emeses/24 hrs; and +2, recurrent emesis). Drowsiness (0–2) and extrapyramidal signs were reported as such. Other potential side effects such as akathisias, insomnia, and diarrhea were also to be reported.

RESULTS

A total of 63% of the patients did not experience any nausea or vomiting, 27% experienced +1 nausea and vomiting on the third day of treatment, and three patients only (10%) experienced significant recurrent vomiting. The latter three patients had mild symptoms starting on the second day of therapy and developed severe symptoms following the addition of doxorubicin and cyclophosphamide on the third day of chemotherapy. Extrapyramidal side effects (16%) consisting of dystonic reactions and akathisias necessitated increase in the dose frequency of DPH and simply slowing down the MCP infusion (table 2). No increase in the incidence of nausea and vomiting was noticed during subsequent cycles of therapy in every patient studied, and no incidence of renal failure (> 1.3 mg/dl) was noted following each cycle administered.

TABLE 2.—Efficacy and side effects of treatment

	No. of patients (%)		
	Day 1	Day 2	Day 3
Emesis			
0	30 (100)	27 (90)	19 (63)
+1	0 (0)	3 (10)	8 (27)
+2	0 (0)	0 (0)	3 (10)
Extrapyramidal symptoms		2 (7)	5 (16)
Drowsiness			
+1	30 (100)	30 (100)	30 (100)
Insomnia		2 (7)	2 (7)

DISCUSSION

The advantage of MCP over other antiemetic agents has carefully been documented in randomized double blind studies (1–3) with approximately 40% of patients not experiencing any emesis. However, all the trials reported so far described its antiemetic role when given in intermittent doses starting minutes prior to a single cisplatin infusion and continuing intermittently as well for a few hours postinfusion. The latter infusion would last from only a few minutes in some studies up until 6 hours in other studies.

We, like others, recently started to use high-dose (daily) cisplatin infusions. To control severe emesis, we implemented continuous MCP infusion prior to and throughout the 3-day infusional therapy. We also recommended extension of the antiemetic treatment for at least 12–18 hours following the last cisplatin infusion because of our prior unpublished experience and experience of others (4,5) of significant emesis if MCP were to be stopped earlier.

In our experience, the cisplatin-containing combination used in this study is highly emetogenic when only conventional doses of MCP (0.5 mg/kg/day) are used (> 90% incidence of emesis, unpublished observations). This is in keeping with observations made by others (1,2) and common experience as well. Our study highlights the safety of prolonged MCP infusion when given in a continuous dose of 2 mg/kg/24 hours for a total of 84–90 hours. DPH was added as an antidote for potential extrapyramidal side effects of MCP. Our study also demonstrates the effectiveness of emesis control by the antiemetic combination administered, since 63% of the patients studied had no emesis induced by the cisplatin-containing multidrug regimen used.

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BRIEF REPORTS

Weekly Cyclophosphamide and Alternate-Day Prednisone: An Effective Secondary Therapy in Multiple Myeloma[1,2]

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For the Clinical Trials Group of the National Cancer Institute of Canada

Intravenous or oral cyclophosphamide, 150–250 mg/m^2 (500 mg maximum), once per week with alternate-day oral prednisone, 100 mg, was given to 57 myeloma patients resistant to melphalan and prednisone (MP). Seven responses in 28 primary MP-resistant patients and ten responses in 29 secondary MP-resistant patients were observed. Previous response to MP was not a significant factor in predicting response to weekly cyclophosphamide and alternate-day prednisone. The results suggest that the regimen of weekly cyclophosphamide and alternate-day prednisone may be as effective as more aggressive regimens in the treatment of patients with myeloma who have failed MP therapy. However, a randomized trial would be required to determine the relative contributions of cyclophosphamide and prednisone to the effectiveness of this regimen. [Cancer Treat Rep 71:981–982, 1987]

Since 1973, the Clinical Trials Group of the National Cancer Institute of Canada (NCIC) has completed two studies of chemotherapy in multiple myeloma. Low response rates to monthly cyclophosphamide and prednisone in primary melphalan plus prednisone (MP)-resistant patients and to combination chemotherapy with doxorubicin, lomustine, vincristine, and prednisone in secondary MP-resistant patients and a preliminary report of weekly cyclophosphamide and alternate-day prednisone (CP) (1) led us to study this regimen further in melphalan-resistant myeloma.

MATERIALS AND METHODS

A total of 57 evaluable patients, median age, 61.7 years, 79% of whom had Karnofsky performance status >50% (43 stage III, 13 stage II, and one stage I disease), received cyclophosphamide, 150–250 mg/m^2 (total single dose $\leq$ 500 mg), iv once per week along with prednisone, 100 mg orally, on alternate days. If the iv route was not practical, oral cyclophosphamide in the same doses was given. Pancytopenic patients received the lower dose of cyclophosphamide and dose escalation by 50 mg/m^2 per week up to 250 mg/m^2 (500 mg maximum) was undertaken.

Patients failing treatment with MP were divided into two groups; those who never achieved a stable response to MP (primary resistance) and those who achieved a stable response and then relapsed, despite continuing or reinstituting MP (secondary resistance). Criteria of stable response were (*a*) reduction in the serum M protein to < 50% of the baseline concentration, (*b*) reduction in paraproteinuria to < 0.3 g/24 hours or to < 90% of the baseline value, and (*c*) shrinkage of measurable plasmacytoma by > 50%. Stable responses were of minimum 4 months' duration.

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Criteria of response for patients receiving CP were the same as those for melphalan with the exception that a minimum duration of only 2 months was required. Inadequate responses included patients with either no objective change for at least 12 months or improvement insufficient to qualify as an objective response. Patient data were collected and analyzed by the central office of the Clinical Trials Group and accuracy of paraprotein recording was ensured by obtaining copies of the electrophoretic strips.

RESULTS

In 28 patients who did not have a stable response to MP, seven (25%) responses were seen lasting 2–20 months (mean, 9). Of 29 patients who responded with stabilization on MP, ten (35%) showed further response (mean duration, 10.5 months) on CP, five (17%) had inadequate responses (mean duration, 10 months), and 14 (47%) had progressive disease. The overall response rate was 30%. The 95% confidence interval around this response rate is 18%–42%. The likelihood of response was unrelated to previous response to MP ($P = 0.56$), performance status, stage, extent of bone disease, degree of anemia, hypercalcemia, uremia, or type of paraprotein. Of 23 patients who either responded or showed some improvement, cyclophosphamide was given only intravenously in 12, only orally in seven, and by both routes in four patients. Of three patients who did not receive prednisone along with weekly cyclophosphamide, one response occurred for 9 months in a patient with previous stable MP response, and two patients had progressive disease. Prednisone was discontinued after 3 days due to stomach upset in another patient who achieved a response for 5 months with weekly cyclophosphamide, having had an inadequate response to MP. Septic neutropenic death occurred in one patient who refused antibiotic treatment while responding within 5 weeks of starting CP. One patient developed acute myeloblastic leukemia after receiving 3.93 g/m^2 of cyclophosphamide and 847 mg/m^2 of melphalan. Cyclophosphamide was discontinued because of dermatitis in one patient who had subsequent stable disease for 31 months with carmustine therapy.

Median survival for all patients from starting CP therapy was 27 weeks. Median survivals from starting CP according to response were 4 months for those who progressed, 10.5 months for those who had no change or minimal improvement, and 12 months for those who responded.

DISCUSSION

Weekly cyclophosphamide and alternate-day prednisone have yielded similar results to those obtained with more complex regimens for MP-resistant myeloma. Most previous reports of second-line chemotherapy in myeloma have involved small series of patients and concluded that primary MP-resistant patients have fewer responses than those with secondary resistance (2). However, the response rate with CP was not significantly lower in patients with primary resistance to melphalan although there was a trend in favor of higher response to CP in previous MP responders.

The results of weekly CP therapy must be interpreted in the light of increasing recognition of the cytotoxic role of prednisone (3). Patients receiving CP had in effect a dose escalation of prednisone, receiving 350 mg/week compared with an equivalent weekly dose of 100 mg for patients on monthly MP. Pulse prednisone with equivalent weekly doses of about 200 mg has yielded a 31% response rate with symptomatic and survival benefits compared with nonresponding patients (4). It is likely that escalated prednisone dosage in the CP regimen has contributed to the responses observed. However, the observations of one response in three weekly cyclophosphamide-treated patients who did not receive prednisone at all and one response in a patient who received only two doses of prednisone suggest that the schedule of cyclophosphamide administration may be important.

The importance of dose and schedule of melphalan therapy in myeloma is well established (5) and limited experience suggests that these factors may be important with cyclophosphamide (1,6). Increasing the dose of cyclophosphamide to 2400 mg/m^2/month has been reported to overcome melphalan resistance in 28% of patients with primary resistance and 46% of patients with secondary resistance (6). The dose intensity (7) of cyclophosphamide in the CP regimen (150–250 mg/m^2/week) was considerably less than the 2400-mg/m^2/month regimen described above and could probably be increased in further studies, although this may also increase toxicity. A randomized trial would be equired to determine the relative contributions of cyclophosphamide and prednisone to the effectiveness of the CP regimen.

In conclusion, this regimen of CP therapy is well tolerated and is a practical alternative to more complex, toxic, and costly regimens for the treatment of MP-resistant myeloma.

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Open-Label Phase II Trial of Recombinant Beta Interferon (IFN-beta$_{ser}$) in Patients With Colorectal Cancer[1]

Pierre L. Triozzi, Patricia Kenney, Donn Young, and John J. Rinehart[2,*]

Phase II clinical trials with interferon (IFN)-alfa have proven that this agent has no significant antitumor activity in colorectal adenocarcinoma (1). IFN-beta has been demonstrated to have greater antiproliferative activity and different tissue distribution than IFN-alfa in vitro (2). Phase I clinical trials using IFN-beta$_{ser}$, a synthetic muteine IFN-beta, have noted a maximum tolerated dose in the range of 100–300 megaunits iv, ie, tenfold higher than IFN-alfa (3). Therefore, an open-label phase II study of IFN-beta$_{ser}$ in patients with colorectal cancer was undertaken.

METHODS

Patients with histological confirmation of colorectal cancer at The Ohio State University (OSU) Hospitals were eligible if the following criteria were met: no prior chemotherapy; Eastern Cooperative Oncology Group performance status ≤ 2; leukocyte count $> 4000/mm^3$, platelet count $> 100{,}000/mm^3$, hemoglobin > 9 g/dl; BUN < 25 mg/dl; creatinine < 1.5 mg/dl; bilirubin < 1.5 mg/dl; SGOT and alkaline phosphatase $< 2 \times$ normal; and the presence of bidimensionally measurable disease (hepatic lesions were measured by computer tomography). Signed informed consent was required, and the protocol was approved by the OSU Human Subjects Review Board.

IFN-beta$_{ser}$ was provided by the Triton Biosciences Corporation (Alameda, CA). Thirteen patients received 90 megaunits by iv bolus on Monday, Wednesday, and Friday during each week for 4 weeks. Four-week cycles were repeated three times or until excessive toxicity or progression of disease occurred. Toxicity was assessed using World Health Organization (WHO) criteria (4). The occurrence of grade III or IV toxicity resulted in a 50% dose reduction. If three dose reductions were required, patients were taken off study. Responses were defined using WHO criteria (4).

RESULTS

All 13 patients were evaluable for response (table 1). No objective responses were observed. Toxic effects are shown in table 2. All patients developed fever, chills,

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	13
Male/female	6/7
Evaluable	13
Median age in yrs (range): 57 (37-73)	
Initial performance status	
0	7
1	6
2	1
Prior therapy	
None	12
Radiation therapy	1
Primary site	
Colon	11
Rectum	2
Metastases	
Liver	10
Lung	3
Soft tissue	2
Response	
Complete	0
Partial	0
Minor	0
No change	4
Disease progression	9

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TABLE 2.—Toxicity

Toxic effect	Grade* I	II	III	IV
Hematologic				
Granulocytes	4		1	
Platelets				1
Hepatic				
Bilirubin				3†
SGOT	4	4	1	
Renal				
Gastrointestinal				
Nausea/vomiting	5			
Diarrhea	3			
Fatigue	5	5	2	
Neurologic			1	
Weight loss				

*WHO classification: I = mild; II = moderate; III = severe; and IV = life-threatening.

†All 3 patients had progression of liver metastases.

fatigue, and malaise. One patient developed transient asymptomatic hypotension. Tender gingiva in one patient and nocturia in another were also noted.

Liver function values increased in nine patients. Seven of these patients had liver metastases, and in six, progression of liver metastases was documented. Two patients without other evidence of liver metastases developed grade II liver function test abnormalities; one of these patients did develop acute bowel obstruction.

DISCUSSION

Although well-tolerated, IFN-beta$_{ser}$ at 90 megaunits by iv bolus three times a week produced no objective tumor regression in 13 patients with advanced colorectal carcinoma. The probability of the true response rate being $> 20\%$ is < 0.06. It is conceivable that higher doses of IFN-beta$_{ser}$ and/or more frequent administration may be more efficacious.

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Phase II Evaluation of Spirogermanium in Malignant Melanoma: A Southwest Oncology Group Study[1,2]

J. Wendall Goodwin,[3] Kenneth Kopecky,[4] Milan Slavik,[3] Bill L. Tranum,[5] Stanley P. Balcerzak,[6] William S. Fletcher,[7] and John J. Costanzi[8,*]

Malignant melanoma currently accounts for 1% of all cancers. Current agents induce an approximate 21% response rate, with a median duration of response < 1 year (1). More active compounds must be sought.

Spirogermanium is an azaspirane with activity in some animal tumor systems (2–4). This phase II study was undertaken to evaluate the activity of spirogermanium in malignant melanoma.

METHODS

Twenty patients with Southwest Oncology Group performance status ≥ 3 with histologic diagnosis of advanced malignant melanoma were entered in a phase II study with only one prior treatment allowed. Ten good-risk patients (median age, 51 years) received 120 mg/m^2 three times weekly for 2 weeks, then twice weekly for 2 weeks, then weekly thereafter. Poor-risk patients included those who were > 65 years of age (median, 71) or who had poor tolerance to prior chemotherapy. These patients received 80 mg/m^2 following the same schedule. There were schedules for dose escalation and standard response criteria were used.

RESULTS

Patient characteristics are summarized in table 1. Seventeen patients received prior therapy, most commonly with a combination containing dacarbazine (15). Four of 17 patients (23.5%) responded to this initial therapy. There were two early deaths, while one patient refused further treatment after 1 week. The remaining 17 patients had tumor progression at a median of 4 weeks (3 weeks, poor-risk patients; 5 weeks, good-risk patients) and died at a median of 11.5 weeks (11 weeks, poor-risk patients; 13 weeks, good-risk patients). Since there were no responses among the 18 patients evaluable for response, the one-sided upper 95% confidence limit for response is 15%. Poor-risk patients tended to be diagnosed within 1 year of registration, while good-risk patients tended to be diagnosed > 2 years from registration.

Toxicity was generally moderate to mild and consisted chiefly of nausea and vomiting (nine patients), neurologic (one severe, three moderate, and three mild), and thrombocytopenia (one life-threatening, one severe, and one moderate). Of those patients with thrombocyto-

TABLE 1.—Patient characteristics

Characteristic	No. of— Good-risk patients	Poor-risk patients
Total	10	10
Male:female	8:2	9:1
Median age in yrs (range)	51 (25–64)	71 (47–83)
Prior chemotherapy		
Yes	9	8
No	1	2
Organ involvement		
Distal lymph nodes	4	1
Bone	5	2
Brain	3	1
Liver	1	4
Lung	3	6

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penia, two had had prior dacarbazine, while the patient with life-threatening thrombocytopenia had had no prior therapy but had marrow involvement.

CONCLUSION

In this phase II study of patients with one prior treatment or less, spirogermanium exhibited no antitumor activity. The incidence of thrombocytopenia in further studies utilizing spirogermanium alone or in combination with other agents needs to be strictly monitored.

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Second-Line Chemotherapy With Tauromustine in Metastatic Breast Cancer in Postmenopausal Women: A Phase II Study[1]

Peter Ernst,* Ingegerd Balslev, and Henning T. Mouridsen[2]

Tauromustine (TCNU) is a nitrosourea compound in which the nitrogen group of taurine constitutes one of the nitrogens. Furthermore, the sulfonic acid group of taurine is transformed into a dimethylaminosulfonyl group. Therapeutic activity of TCNU in breast cancer has been observed in a phase I study (1). Marrow suppression and gastrointestinal toxicity are dose-limiting, and the recommended dose for phase II trials ranges from 90 to 150 mg/m^2 every 4–5 weeks, depending on the extent of previous antineoplastic therapy or irradiation (1).

The present phase II study analyzes the efficacy of TCNU administered as second-line cytotoxic therapy to postmenopausal women with advanced breast cancer.

MATERIALS AND METHODS

Postmenopausal patients ≤ 70 years old with histologically proven, locally advanced and/or metastatic breast cancer were eligible. Other criteria of eligibility were measurable and/or evaluable progressive disease, performance status ≤ 3, clinical resistance to prior combination chemotherapy, and normal renal and bone marrow functions. Informed consent was obtained.

TCNU was administered orally every 5 weeks at a dose of 100 mg/m^2. The initial dose was reduced to 80 mg/m^2 for patients having received prior extended-field irradiation to axial skeleton. The dose of TCNU was adjusted according to platelet and wbc counts 5 weeks after the previous course.

Treatment was continued until progressive disease or until the occurrence of severe side effects.

Assessment of response, duration of response, and time to treatment failure were defined according to International Union Against Cancer criteria (2). Toxic effects were graded according to World Health Organization (WHO) criteria (3).

RESULTS

Twenty-nine consecutive patients were entered in the study. Four patients were considered ineligible and two patients were lost to follow-up. Thus, response and toxicity could be evaluated in 23 patients. The median age was 53 years (range, 41–69). Median performance status was 1 (range, 0–3), and prior chemotherapy with or without tamoxifen for advanced disease included CMF (cyclophosphamide, methotrexate, and 5-fluorouracil) in eight patients, CAF (cyclophosphamide, doxorubicin, and 5-fluorouracil) in nine patients, and PNMF (prednimustine, novantrone, methotrexate, and 5-fluorouracil) in six patients.

Progressive disease was observed in 18 patients including three with early death (ie, before 10 weeks) from malignant disease. Three patients had no change, persisting for 3, 3, and 6 months. In one patient a partial response lasting 7+ months and in another patient a complete response lasting 8 months were observed. The median time to disease progression was 2 months.

Fifty courses of TCNU were administered, and in 41 courses the patient could be followed for toxic effects for the scheduled 5 weeks. These are shown in table 1.

TABLE 1.—Toxic effects*

	WHO grade					
	0	1	2	3	4	Total
Leukopenia	24	6	9	2	0	41
Thrombocytopenia	24	9	4	2	2	41
Nausea/vomiting	1	23	12	5	0	41
Diarrhea	37	3	0	1	0	41
Sweating	34	3	3	1	0	41
Tumor pain	26	4	11	0	0	41

*Values = No. of courses.

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Wbc and platelet count nadirs were observed in the 4th and 5th weeks after treatment, with occasional protracted marrow suppression (8 weeks). Altogether, the toxic effects from TCNU led to dose reduction and/or postponement of therapy in 51% of the patients, and in only one patient was dose escalation possible.

DISCUSSION

In the present study response was demonstrated in only two of 23 evaluable patients, and the overall time to treatment failure was 2 months. Although all of the patients had received prior combination chemotherapy for advanced disease, the results indicate that the efficacy of TCNU is comparable to that of the original nitrosoureas (4). The drug has significant marrow toxicity and side effects that prohibited dose escalation. Thus, TCNU plays no role in second-line chemotherapy of breast cancer.

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Carboplatin in Advanced, Refractory Germ Cell Neoplasms: A Phase II Eastern Cooperative Oncology Group Study[1,2]

Donald L. Trump,* Paul Elson, Harvey Brodovsky, and Steven E. Vogl[2]

With the development of cisplatin-based chemotherapy for advanced germ cell tumors it is possible to cure 70%–80% of all patients with metastatic disease (1–3). There is still clearly a need for improved initial and salvage therapy. Consequently, the Eastern Cooperative Oncology Group (ECOG) conducted this phase II trial of carboplatin, the cisplatin analogue, (1, 1 diammine cyclobutanedicarboxylatoplatinum II) in advanced, refractory germ cell neoplasms.

METHODS

Patients Studied

Twenty-two patients with measurable, progressive advanced germ cell tumors were entered on this trial between January 1984 and April 1986. ECOG performance of 0, 1, 2, or 3, and adequate bone marrow (wbc count $> 4000/mm^3$, platelet count $> 100,000/mm^3$), renal (creatinine < 1.6 mg/dl), and hepatic function (bilirubin < 1.5 mg/dl) were required.

Treatment Administration

A total of 400 mg/m^2 of carboplatin was administered as an iv infusion over 20–30 minutes every 28 days. Patients who had received radiation therapy to either the whole pelvis or thoracic or lumbar spine were given an initial dose of 320 mg/m^2.

Patients were to receive at least two cycles of therapy unless progressive disease was noted. Patients with stable or responsive disease were to continue therapy until progression. ECOG criteria for toxicity and response were utilized.

RESULTS

All patients were evaluable for toxicity and response (table 1). Tumors in 20 of 22 patients progressed during therapy with carboplatin without undergoing a partial or complete response to therapy. One patient was removed from study because of persistence of leukopenia (< 2500 cells/mm^3) 6 weeks following the first dose of carboplatin. One patient may have benefitted from carboplatin. This patient had pulmonary metastases from a seminoma. He had had a partial response in pulmonary disease following vinblastine, bleomycin and cisplatin and doxorubicin, cyclophosphamide, and etoposide. At the time of entry into this trial, multiple pulmonary nodules were present which had been stable for 1 month. Serum beta human chorionic gonadotropin and alpha fetoprotein had always been normal. The patient received three cycles of carboplatin; further regression of pulmonary nodules occurred and a partial response was documented. Six weeks after the third treatment with carboplatin a left thoracotomy was done. Six nodules were resected. Histologic examination of all six resected nodules revealed only necrosis and fibrosis. Residual nodules in the right lung were also judged likely to be composed of necrosis and scar.

Survival of these patients was short. Median survival was 3.4 months; two patients are alive 13 and 21 months after initiation of carboplatin.

Myelosuppression was the most serious toxic effect noted in this trial. There was a 14% incidence (three patients) of life-threatening thrombocytopenia ($< 25,000$ cells/mm^3) and an additional 27% incidence (six patients) of severe thrombocytopenia (25,000–49,999 cells/mm^3).

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[2] This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in the study: University of Wisconsin Clinical Cancer Center (*D. L. Trump*, CA-21076); Dana-Farber Cancer Institute, Harvard School of Public Health, Boston, MA (*P. Elson*, CA-23318); Fox Chase Cancer Center, Philadelphia, PA (*H. Brodovsky*, CA-18281); Albert Einstein College of Medicine, Bronx, NY (*S. E. Vogl*, CA-14958). Other participating institutions include: Medical College of Ohio, Toledo; New York University Medical Center, New York, NY (CA-16395); Northwestern University Medical Center, Chicago, IL (CA-17145); Hospital of the University of Pennsylvania, Philadelphia (CA-15488); University of Pittsburgh, PA (CA-18653); University of Rochester Cancer Center, NY (CA-11083); Roswell Park Memorial Institute, Buffalo, NY (CA-12296); University of Pretoria, South Africa (CA-21692); SUNY-Downstate Medical Center, Brooklyn, NY; Tufts University, Walpole, MA (CA-07190); and Twin Tiers CCOP, Binghamton, NY.

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TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)	Median survival (mos)
Initial performance status		
0, 1	16 (73)	4.2
2, 3	6 (27)	1.5
Total	22	3.4
Median No. of prior cycles of cisplatin-based therapy (range): 8 (1-22)		
Best response to prior therapy		
Complete response	6 (6 mos median duration)	
Partial response	16	
No. of cycles of carboplatin		
1	7 (32)	
2	11 (50)	
≥ 3	4 (18)	
Response		
Progression	20	
Unevaluable	1	
Complete response	1 (95% confidence interval = 0.001-0.228)	

One patient experienced a wbc count nadir of $< 2000/mm^3$. Only one patient was noted to have severe nausea and vomiting following carboplatin administration. All other toxic effects were mild; no drug-related deaths occurred.

DISCUSSION

While comparative clinical data are limited, preclinical information for animal tumor systems in vivo and in vitro as well as human tumor xenografts and human tumor stem cell assay data indicate that substantial cross-resistance exists between cisplatin and carboplatin. Motzer et al (4) have reported partial responses in two of 22 patients with resistant germ cell tumors treated with carboplatin. Whether Motzer's patients are comparable to our patients in terms of prior cisplatin therapy is not clear. The 95% confidence intervals for the response rates in the present study and Motzer's trial do overlap.

Carboplatin is not useful in the salvage therapy of patients with germ cell tumors resistant to cisplatin. Higher doses, use earlier in the course of disease, and use of carboplatin and cisplatin are approaches under investigation (5).

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Idarubicin in the Treatment of Relapsed or Refractory Acute Myeloid Leukemia

Idarubicin (IDR) is a new anthracycline derivative which differs from daunorubicin (DNR) by the replacement of the methoxy group in position 4 with a proton (1). IDR is five times more potent than DNR against L1210 and P388 murine leukemias and is significantly less cardiotoxic than DNR in various animal species (2). In man, preliminary phase I and II studies suggested that IDR was effective in treating acute leukemia when used in a 3-day regimen (8–15 mg/m^2 iv) (3,4). In France, we decided to study a 5-day regimen to obtain a longer exposure of the leukemic cells to the drug. A multicenter phase II study was initiated, with the participation of 14 French institutions.

PATIENTS AND METHODS

Based on the results of a preliminary phase I study (5), the starting dose was 7 mg/m^2/day × 5 (rapid iv infusion): 29 patients received this regimen. Since the extrahematologic tolerance was good, the daily dose was escalated to 8 mg/m^2: 26 patients received this second regimen.

Patients > 15 years of age were considered eligible to participate in the present study if they were diagnosed as having acute myeloid leukemia (AML) or chronic myeloid leukemia in myeloid blast crisis (CML-BC), with at least 30% blasts in the bone marrow.

Criteria for exclusion were a limiting vital organ toxicity or dysfunction: serum bilirubin greater than four times the normal value, serum creatinine > 130 μmols/L, and history of congestive heart failure (CHF).

Fifty-five patients were entered in this study. Their clinical characteristics are shown in table 1. Eight patients had secondary leukemia (chemotherapy- and/or radiotherapy-induced) and had not been previously treated for their leukemia. In three of five patients with CML-BC, IDR was given as first-line chemotherapy. Forty-two patients had previously received conventional induction protocol including an anthracycline (DNR or zorubicin). Twenty-eight patients with AML received IDR as first treatment of their first relapse, occurring after 2–57 months of remission (median, 7 months). Among the 14 patients with advanced AML, five had never achieved remission, three were in first refractory relapse, and six were in second relapse.

TABLE 1.—Clinical characteristics of 55 patients treated with idarubicin

Characteristic	7 mg/m^2	8 mg/m^2
Total No. of patients	29	26
Median age in yrs (range)	50 (21–77)	56 (18–74)
Sex (male/female)	16/13	11/15
Diagnosis (No. of CRs)		
Secondary AML	6 (2)	2 (0)
AML first relapse	12 (2)	16 (5)
Advanced AML	7 (1)	7 (1)
CML-BC	4 (0)	1 (0)
Wbc count (× 10^9/L)		
Median	4.7	6
Range	1–460	0.5–160
Platelet count (× 10^9/L)		
Median	58	49
Range	10–677	2–141
No. of patients previously treated with anthracycline	20*	22*
Prior cumulative dose of daunorubicin (mg/m^2)		
Median	380	400
Range	135–900	35–600

*+ 1 patient treated with aclarubicin.

RESULTS AND DISCUSSION

Of the 55 patients, six died from infectious complications (four early deaths and two nonblastic aplasias). They were not excluded from the analysis and were considered failures.

Eleven patients achieved complete remission (CR), five at 7 mg/m^2 (one after two courses) and six at 8 mg/m^2 (one after two courses). Six additional patients had partial remission (PR). Among eight secondary leukemias, two CRs and one PR were recorded. There were two CRs and one PR in the group with advanced AML: one CR and one PR among six patients in second relapse, and one CR among eight patients with refractory leukemia. One PR was achieved in five patients with CML-BC. The best results were obtained in the group with AML in first relapse with seven CRs (25%) and three PRs among 28 patients. In this group, the CR rate was somewhat higher at 8 mg/m^2/day (31% vs 17% at 7 mg/m^2/day). At the dose of 7 mg/m^2/day, 11 of 29 patients experienced nausea and five had mild vomiting. Diarrhea was recorded in ten patients and was severe in only one. Mucositis was noted in seven patients and compromised oral intake in five of them. Abnormalities in liver function tests were infrequent (four

patients) and moderate in severity. No CHF or significant arrythmias were seen. In one patient who had previously received 440 mg/m^2 of DNR, the isotopic left ventricular ejection fraction fell from 52% pretreatment to 38% posttreatment without clinical manifestations. At the higher dose (8 mg/m^2), the main problem was mucositis which compromised oral solid intake in five patients. Nausea and vomiting were frequent but only two patients required specific antiemetic treatment. Diarrhea, which had been the limiting side effect of escalating the dosage in phase I (5), was noted in seven patients and was severe in only one patient. No CHF was seen. In one patient who had previously received 480 mg/m^2 of DNR, echographic ventricular ejection fraction fell from 63% pretreatment to 40% posttreatment. In one patient who had received 400 mg/m^2 of DNR transient ECG changes were noted. This study confirms the efficacy of IDR in AML since 11 of 50 patients (22%) achieved CR and five achieved PR (10%). In previous phase II studies IDR was used in 3-day regimens. With 8 mg/m^2/day Carella et al (4) obtained two CRs among eight refractory patients and one CR among ten patients with AML in first relapse. With 10–15 mg/m^2/day, Daghestani et al (3) reported an 18% CR rate among 28 previously treated patients with AML. The 5-day regimen appears to be well-tolerated even at 8 mg/m^2/day and in elderly patients. This schedule could be used in combination with cytarabine in the treatment of AML.

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Mitoxantrone Extravasation Injury

A serious risk of iv administration of cytotoxic drugs is the occurrence of extravasation into the soft tissues which may result in a devastating tissue necrosis (1). Notorious drugs, in this respect, are the anthracyclines, mitomycin, mechlorethamine, and the vinca alkaloids (2).

Recently, Dorr et al (3) reported results from a study where the drugs cisplatin, melphalan, and mitoxantrone were tested for vesicant activity in mice by giving intradermal injections equivalent to human clinical doses, calculated on a body surface area basis. Neither cisplatin nor melphalan nor mitoxantrone caused any skin necroses and they concluded that in clinically relevant doses these cytotoxic agents are not vesicants (3). Up to now, clinical experience with mitoxantrone extravasations also seems to indicate that the drug produces no undue toxic effects (4,5). However, in our clinical practice we were confronted with a case of severe, prolonged cellulitis after extravascular leakage of this cytotoxic agent. The clinical course is described.

CASE REPORT

A 72-year-old woman with a history of carcinoma of the breast appeared to have progressive disease along with malignant pleuritis and pericarditis. In 1971, at the age of 57 years, she underwent a right-sided mastectomy with axillary lymph node dissection.

In 1984 she was treated with tamoxifen, because of metastases in the lymph nodes, and with radiotherapy to some regions.

In May 1985 malignant pleuritis and pericarditis were diagnosed and treatment with mitoxantrone (20 mg iv every 3 weeks) was started. During the third course given iv on the dorsum of the right hand, there was an extravasation of an exiguous amount of the mitoxantrone solution. This resulted in a painless swelling; no special treatment was given. One month later the right-hand dorsum showed a painful red edematous area with crustae. The interval of drug administration, now done on the left hand, was extended to 4 weeks. Two months after the extravasation the lesion developed necrotic areas. Since there was tumor response the treatment was continued. Three months after the injury the ulcer measured 4 × 6 cm and was painful; this necessitated a local excision of the necrotic tissue. The eighth course was reduced to 18 mg; treatment was then discontinued. At this stage the patient had achieved a partial remission but the ulcer on the right hand expanded.

Two months after the last dose the wound was granulating due to local treatment with Eusol-paraffin dressings. Plastic surgery, although planned, could then be cancelled.

Four months after the discontinuation of mitoxantrone and 10 months after the extravasation, the wound was healed.

DISCUSSION

So far, experience in the clinic with accidental mitoxantrone extravasation shows no complications (4,5). This is surprising, taking into consideration the resemblances between the mechanism of action in inducing cytotoxicity of doxorubicin and its congeners and mitoxantrone. However, in our clinic we noticed a case of severe localized necrotic reactions after the drug was inadvertently extravasated. The extravascular leakage may have been provoked by a temporarily high local mitoxantrone blood concentration after administration, due to a poor blood drain in the right arm caused by the right-sided mastectomy. This may also have contributed to the delay of the healing process. The failure of spontaneous healing in our case may also be related to the continuation of mitoxantrone chemotherapy, justified by the patient's tumor regression, which has resulted in delivery of the cytotoxic drug in the area of the lesion. Cessation of chemotherapy and local treatment with Eusol-paraffin resulted, finally, in a complete recovery of the skin.

With this case report we would like to call attention to the fact that mitoxantrone presents a distinct hazard after extravasation, contrary to observations in earlier studies.

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Frans T. M. Peters*
Jos H. Beijnen
Wim W. ten Bokkel Huinink[1]

June 30, 1987

[1] Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands.

* *Reprint requests to:* Frans T. M. Peters, MD, Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

Unexpected Toxicity Encountered in the National Wilms' Tumor Study

The fourth National Wilms' Tumor Study (NWTS) has been randomizing low-risk patients between study arms that include "standard" dactinomycin (AMD) administration and a pulsed/intensive (P/I) course. The low-risk group is defined as children with localized disease (stages I and II) and tumors with favorable histology (FH) features. The doses were 15 μg/kg/day for 5 days and 60 μg/kg once, respectively. Both regimens include vincristine (VCR) given at doses of 1.5 mg/m^2 at weekly intervals for ten treatments. The standard arm received another cycle of AMD at Week 5, and this was repeated at Week 13 when two additional doses of VCR were given. The P/I regimen delivers AMD at 3-week intervals through Week 15; at Weeks 12 and 15, the VCR is given at single doses of 2 mg/m^2. These regimens are for the stage I children with FH features and are identical but extended in time for stage II/FH patients. Five of 40 stage I and II children randomized to receive the P/I regimens have developed signs of profound hepatotoxicity. Hepatotoxicity is manifested as marked elevations in the values of the liver function tests, ascites, and liver enlargement. One of the four children with other complicating factors died. The other four children appear to have recovered satisfactorily. No similar observations have been made in the standard arms, nor has this problem been prominent in the previous experience of the NWTS which has been operational with the standard regimens for 18 years.

It would appear on review of the five children reported to the Committee that each had one or more contributory factors in the clinical management or the clinical course, for example, repeat anesthesia. The initial impression is that the liver will tolerate the P/I course at 60 μg/kg so long as no additional demands are made on the liver; in that case, hepatotoxicity may develop.

The NWTS Committee, having reviewed each individual patient in great detail, has therefore changed the single dose of AMD in the P/I arm from 60 to 45 μg/kg.

We wish to bring this matter to the attention of all practicing oncologists who may be considering a change in the schedule of AMD administration from multiple daily doses to single injections.

Giulio J. D'Angio[1,*]

For the NWTS Committee

July 1, 1987

[1] Children's Cancer Research Center, Philadelphia, PA.

* *Reprint requests to:* Giulio J. D'Angio, MD, Department of Radiation Therapy, Hospital of the University of Pennsylvania, 3400 Spruce St, Philadelphia, PA 19104.

ANNOUNCEMENTS

First International Breast Cancer Chemoprevention Workshop-Conference

New York, New York, November 20, 1987

For further information, contact: Jaclyn Silverman, Chemoprevention Workshop Coordinator, Division of Medical Oncology, Mount Sinai Medical Center, One Gustave L. Levy Place, New York, NY 10029 (telephone: 212-369-5440 or 213-650-6772).

III Biennial Conference of the Indian Society of Oncology

Bangalore, India, February 10–12, 1988

For further information, contact: M. Krishna Bhargava, Director, Kimio and Chairman, Organizing Committee, Iso, Kidwai Memorial Institute of Oncology, Hosur Rd, Bangalore-560 029, India.

Clinical Hematology and Oncology: 1988

San Diego, California, February 15–17, 1988

For further information, contact: Bonny Mower, Department of Academic Affairs, Box 400S, Scripps Clinic and Research Foundation, 10666 N Torrey Pines Rd, La Jolla, CA 92037 (telephone: 619-457-8556).

Second International Congress on Neo-Adjuvant Chemotherapy

Paris, France, February 19–21, 1988

For further information, contact: Service d'Oncologie Médicale, Professeur Claude Jacquillat, 47, boulevard de l'Hôpital, 75651 Paris Cedex 13, France.

Third International Conference on Adjuvant Therapy of Breast Cancer

St. Gallen, Switzerland, March 3–5, 1988

For further information, contact: Prof. Dr. Hans-Jörg Senn, Oncology Center, Department of Medicine C, Kantonsspital, CH-900 F St. Gallen, Switzerland (telephone: 71-26-10-62).

Arizona Cancer Center 6th Winter Symposium: Advances in Hematologic Malignancies and Bone Marrow Transplantation

Snowbird, Utah, March 5–12, 1988

For further information, contact: Mary Humphrey, Conference Coordinator, Arizona Cancer Center, Tucson, AZ 85724 (telephone: 602-626-2276).

American Radium Society Seventieth Annual Meeting

Seattle, Washington, April 16–20, 1988

For further information, contact: Ms. Suzanne Bohn, Administrative Director, American Radium Society, 1101 Market St, 14th Floor, Philadelphia, PA 19107 (telephone: 215-574-3179).

8th Annual Meeting of the North American Hyperthermia Group

Philadelphia, Pennsylvania, April 16–21, 1988

For further information, contact: Radiation Research Society, 1101 Market St, 14th Floor, Philadelphia, PA 19107 (telephone: 215-574-3153).

36th Annual Meeting of the Radiation Research Society

Philadelphia, Pennsylvania, April 16–21, 1988

For further information, contact: Radiation Research Society, 1101 Market St, 14th Floor, Philadelphia, PA 19107 (telephone: 215-574-3153).

Fifth World Conference on Lung Cancer

Interlaken, Switzerland, August 28–September 1, 1988

For further information, contact: Rudolf A. Joss, MD, Secretary General, Institute for Medical Oncology, Inselspital, CH-3010 Bern, Switzerland.

2nd International Meeting of the Metastasis Research Society

Stadhalle, Heidelberg, Federal Republic of Germany, September 26–29, 1988

For further information, contact: Professor V. Schirrmacher, Institut fur Immunologie und Genetik, Deutsches Krebforschungszentrum, Im Neuenheimer Feld 280, D-6900 Heidelberg 1, Federal Republic of Germany.

Yearly Meeting of the German Institute for Laser Medicine

Vienna, Austria, October 5–8, 1988

For further information, contact: Doz. Dr. A. Tuchmann, c/o Wiener Medizinische Akademie, Alser Strasse 4, A-1090 Vienna, Austria (telephone: 0222-427165).

13th Congress of the European Society for Medical Oncology

Lugano, Switzerland, October 30–November 1, 1988

For further information, contact: F. Cavalli, MD, Division of Oncology, Ospedale San Giovanni, 6500 Bellinzona, Switzerland.

Workshop on Progress in Regional Cancer Therapy

Vienna, Austria, November 3–5, 1988

For further information, contact: Doz. Dr. R. Jakesz, Doz. Dr. H. Rainer, c/o B. Schick, Abteilung für Experimentelle Chirurgie der I. Chirurgischen Universitätsklinik, Alser Strasse 4, A-1090 Vienna, Austria (telephone: 0222-4800/2301).

Albert Lasker Medical Research Awards Annual Luncheon

New York, New York, November 20, 1987

The winners of the 1987 Lasker Award, Drs. Mogens Schou, Susumu Ponegawa, Philip Leder and Leroy Hood, will describe their work and outline their views on future directions in research in the Albert Lasker Medical Research Awards Lectures, to be given Wednesday, November 18, at The Rockefeller University, New York City.

The winners will receive their awards at the Annual Luncheon given by the Albert and Mary Lasker Foundation on Friday, November 20, at the Sheraton-St. Regis Hotel in New York City. The awards will be formally presented by Mrs. Albert D. Lasker.

For further information, contact: Judith Smith, Albert and Mary Lasker Foundation, 870 United Nations Plaza, New York, NY 10017 (telephone: 212-753-8222).

ONCOLOGY OVERVIEWS

The International Cancer Information Center of the National Cancer Institute announces publication of the following ONCOLOGY OVERVIEWS. ONCOLOGY OVERVIEWS are specialized bibliographies with abstracts, each referencing 150-500 recent publications on a cancer topic of high current interest, drawn from over 3000 sources. Leading researchers in the field covered by each OVERVIEW review and select the most relevant and significant abstracts for each topic, resulting in a tightly focused, quick reference to the most recent cancer literature.

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